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BEHAVIOR AND TAXONOMY OF A CHYMOMYZID FLY *(CHYMOMYZIA AMOENA)*

Henretta Trent Band

ABSTRACT: Molecular genetics studies on the chymomyzids have produced divergent results on their relation to the genus *Drosophila*. Behavior has been used to assess the appropriateness of their inclusion in the genus (MacIntyre and Collier, 1986) or off the drosophilid main stem (Beverley and Wilson, 1984). Laboratory and natural population studies on *Chymomyza amoena* in Michigan and Virginia and observations on multiple species aggregations at natural sites in 1986 and 1987 in Virginia's Allegheny Mountains have been carried out. Wing-waving and foreleg splaying are characteristics of both sexes. In nature, females do not approach males until sexually mature. All population sizes seem small. Studies on *C. amoena* indicate that behavioral phenotypic plasticity exists for all stages: larval feeding substrates, pupation site choice, mating system, egg deposition and oviposition site selection. Behavioral traits shared with the lek *Drosophila* (Hawaiian and Australian), genus *Scaptomyza*, subgenus *Scaptodrosophila*, subgenus *Sophophora* and genus *Lissocephala* among the drosophilids, and the tephritids, otitids and hymenopterans outside the family Drosophilidae suggest that chymomyzids retain characteristics of primitive drosophilids that have undergone selective modification in the evolution of different drosophilid lineages. Significant differences in aggression between Michigan and Virginia *C. amoena* populations support this conclusion. Throckmorton (1962, 1966) anticipated the chymomyzid relation to the drosophilid stem from external and internal anatomical studies. A wood breeding habitat of most forest chymomyzids is also in agreement with recent molecular genetics evidence that fermented fruit breeding evolved later in drosophilid evolution.

The status of the chymomyzids within the family Drosophilidae has become controversial. Systematics based on morphological and behavioral data treats them as a separate genus (Wheeler, 1952, 1981; Hackman et al., 1970; Okada, 1976; Bachli & Rocha-Pite, 1981, 1982; Grimaldi, 1986). Molecular systematics places them in the genus *Drosophila* (Collier & MacIntyre, 1977; MacIntyre & Collier, 1986) or considerably distant from this genus (Beverley & Wilson, 1982, 1984). Molecular systematics is not without critics (Throckmorton, 1977, 1978) or cautionary interpreters (Wilson, Carlson & White, 1977).

The phylogenetic position of the chymomyzids underwent revision earlier in the 1970s. Chromosomally, the group is related to the subgenus *Sophophora* of the genus *Drosophila* (Clayton & Ward, 1954; Hackman et al., 1970; Clayton & Guest, 1986). Comparative internal morphological studies also suggested affinities to the Sophophoran and Hawaiian drosophilids (Throckmorton, 1962, 1966). The separate genus status

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was retained. Hackman et al. (1970) agreed on the chromosomal relationships but suggested deriving the chymomyzids as a separate lineage between the *Sophophora* and the *Scaptodrosophila*, with which larvae share morphological traits. Throckmorton (1975) adopted this proposal.

Behavior is a component of taxonomy for chymomyzid species (Wheeler, 1952; Okada, 1976; Grimaldi, 1986). Adults wing-wave; males are aggressive and use their front feet like boxers; matings are of the assault type (Wheeler, 1952). Grimaldi (1986) describes foreleg splaying for a South American chymomyzid. Behavior may provide clues to the taxonomic status of the group, bearing on old and current controversies. Summaries of drosophilid behavior have been provided in recent books or reviews (Spieth, 1975, 1982; Ehrman, 1978; Grossfield, 1978; Ehrman & Parsons, 1981; Barker & Starmer, 1982; Parsons, 1982; Parsons, 1983; Brncic, 1983; Lachaise & Tsacas, 1983; Lumme & Lakovaara, 1983; Mueller, 1985). They contain little behavioral information on the chymomyzids for which only scattered reports exist (Wheeler, 1952; Watabe, 1985; Band, 1986; Grimaldi, 1986).

Laboratory and natural population studies on *Chymomyza amoena* in Michigan and Virginia and other chymomyzids at higher elevations in Virginia's Allegheny Mountains provide comparisons with other drosophilids. It also enables a beginning behavioral comparison to other insect groups (Alcock, 1979; Thornhill & Alcock, 1983). The relationship of the family Drosophilidae to other insect families has also come into question (Beverley & Wilson, 1982; MacIntyre & Collier, 1986)

MATERIALS AND METHODS

Study Sites

A detailed description of study sites for *C. amoena* has been given in Band (1988a). Behavioral studies made use of the East Jordan, Michigan (lat. 45. 10° N), mid-Michigan (lat. 42. 43° N) and mid-South (Lats. 36.29° N to 37.24° N) populations. Additional information has been derived from St. Louis, Missouri (lat. 38.38° N) populations collected and shipped to East Lansing by the late Harrison Stalker. All photographs made in the laboratory have been made with a Nikon camera; all photographs at Mt. Lake Biological Station (MLBS) have been made with a Pentax.

Briefly, populations for East Jordan, Michigan derive from apples collected from a group of old trees summer 1978 through summer 1981. Mid-Michigan populations for mating, oviposition, breeding season and laboratory studies since 1981 primarily derive from farms west of Lansing (site A) having apple and walnut trees, an East Lansing

neighborhood (site B) having a variety of fruit trees, a farm east of East Lansing (site D) with walnut and other nut and apple trees, a thicket of *Malus coronaria* of unknown age which existed until spring 1987 when it was cleared away. Mid-South Virginia sites for *C. amoena* summer behavioral work include Danville orchard and city sites, a Blacksburg orchard, sites along Rt. 700 near the Biological Station and the apple tree at the Station. Other populations have also come from the MLBS vicinity in Giles County, and Eden, North Carolina.

Additional chymomyzid species have been studied also at the Station (1985) and two other localities near the Station in 1986 and 1987, respectively. Observations followed by collection and identification provide information on species aggregation, niche attractiveness and inter-species interactions. If and when present, *C. amoena* is the only banded wing species. Laboratory work provided further information on *C. aldrichii*.

EXPERIMENTS AND OBSERVATIONS

For *C. amoena*, larval and adult behavior will be treated separately, where appropriate.

Substrate Utilization

Carson (1971) recognized four breeding sites for temporal woodland drosophilids: a) fleshy fruits and fungi; b) sap fluxes; c) decaying vegetation; d) flowers. Cosmopolitan species are typically associated with rotting fruits and vegetables (Ehrman, 1978). Throckmorton (1975) notes that generally *Scaptodrosophila* like substrates in a fresher state. *Chymomyza amoena* as a breeder in multiple fruit and nut substrates and in frass is covered in Band (1988a, b, c), as a larval overwinterer in fruits and nuts in Band & Band (1982, 1984, 1987). Re-creation in the laboratory, using fresh commercial apple, of the lengthy emergence from any one apple/native crabapple collection (Band, 1988a) provides data both on an F_1 and F_2 ability to breed in fresh apple, and lengthy prefertile period. Other oviposition work also provides information on larval ability to develop in unripe frassy and ripe fruits.

Larval Behavior and Pupation Site Choices

Grossfield (1978) presents information on drosophilid larval migratory behavior and puparition. *Scaptodrosophila* larvae skip when leaving the substrate, as do some Hawaiian *Drosophila* (Carson et al.,

1970). *Drosophila melanogaster* larvae typically migrate but may pupate in a variety of locations, on the surface or away from the substrate. Many Hawaiian *Drosophila* pupate in the soil; some scaptomyzoids pupate on the substrate surface, others in situ.

To study pupation site choice, individual crabapples (MI) and apples (VA) having *C. amoena* eggs in 1985 and 1986 were placed over potting soil (vermiculite) and numbers of larvae leaving the substrate versus numbers of larvae remaining with the substrate for pupation were determined by transferring the soil to a beaker of water; numbers of pupal cases floating in the water versus total numbers of adults emerging from the culture were compared (Band, 1988a). To determine the tendency for larvae to pupate on the surface, the 12 walnuts yielding many *C. amoena* adults in November 1984 were later inspected; 33 walnuts gathered at the same time, but kept in an unheated shelter and held overwinter, were inspected in spring 1985 (Band & Band, 1987).

Other species are known to migrate following disturbance. Necessity to transfer *C. amoena* larvae to fresh media due to mold, mites or both stimulates migration, followed by pupation, usually within 24 hours. A photograph of this phenomenon was made in summer 1987.

Natural and Laboratory Populations of C. amoena

Carson et al. (1970), Ehrman (1978), Ehrman & Parsons (1981), Spieth (1975, 1982) and Parsons (1982, 1983) provide information on mating behavior in different drosophilid genera and subgenera. Wheeler (1952) and Grimaldi (1986) give descriptions of some chymomyzid behaviors. Observations for a week at East Jordan and sporadically in mid-Michigan enabled comparison of natural population and laboratory observed behaviors. Apples on which adults were displaying in 1981 and 1982 were collected and inspected. Males and females on apples in 1981 were transferred to laboratory medium and time to oviposition determined. A pair captured in 1982 was transferred to apple and time. to oviposition determined. To determine that fallen apples on which flies were displaying were not feeding substrates, 150 adults were distributed in 3 population bottles, supplied with immature unbroken apples and numbers alive after two days determined. By contrast, 179 apples were collected 13 June 1985, inspected for eggs and all were dissected and scored for presence of internal frass. A windstorm blew down many in May.

To determine that some movements occur in both sexes, females were transferred to a population bottle and their manner of walking observed. To date, attempts to photograph some behaviors (e.g. fighting, foreleg splaying) have been unsuccessful. Other behaviors include simple courtship (if any) prior to assault type mating attempts, capture-in-the-air type matings, female avoidance of "courting" males (Band, 1988a).

Time To Oviposition, Laboratory Data

For both intra- and interpopulation crosses, matings were made with nonaged females and males. Time to oviposition was compared with data from interpopulation crosses made with aged flies and pairs captured in nature (Band, 1988a). In 1986 crosses between Michigan *C. amoena* emerging from apples and Virginia *C. amoena* also emerging from apples were carried out on apples + frass (Band, 1988c). Time to oviposition were compared with interpopulation crosses carried out on laboratory medium.

Oviposition Preference, New Versus Used Substrates:

Females of some species produce pheromones that inhibit others of their species from ovipositing on the same piece of fruit (Thornhill & Alcock, 1983). *Drosophila* females commonly lay eggs where others of their species have oviposited (Mueller, 1985). *Chymomyza amoena* females emerging from native crabapples in May 1985 were allowed to choose between two immature firm apples, matched for size, on one of which two *C. amoena* eggs had been placed. A dish of medium assured continued fertility. Four pairs (females and males) were used in three replicates over a seven-day period (Band, 1988a).

Egg Aggregation in Nature

Two sites near MLBS produced apples in summer 1986 and 1987. Collections were scored for the presence of *C. amoena* eggs and negative binomial k values determined (Band, 1988c). Collections of plums and apples were also recorded for *C. amoena* eggs in Michigan in 1987 (Band, 1988b). Egg aggregation were compared in the two states.

Egg aggregation, for an unknown or little known species, is a compound measure of females to oviposit where others of their species have oviposited and for females to lay more than one egg at a time. Behavioral oviposition diversity exists among drosophilids (Carson et al., 1970; Grossfield, 1978; Mueller, 1985). The numbers of substrates with one versus more than one egg were also compared in 1987.

Aggressive Behaviors

The frequency of aggressive events determined by half-hour observations of small populations (7-12 individuals with at least 3 males present) were compared for Michigan and Virginia populations at one-two days after emergence versus 3-6 days after emergence. The number of encounters between individuals (orienting toward one another), number of fights and number of mating attempts were recorded. If

population bottles were moved prior to the timed observations, populations were given a half-hour to equilibrate before observations were made. At least two localities for each state were included in the replicates.

For crowded cultures, Virginia populations of 15-20 adults were used. Glass population bottles were supplied with a dish of laboratory medium.

For more serious consequences of aggression, a clear plastic box was used into which an apple was placed having one or more holes. A small population (3 males, 2 females) was aspirated into it and timed experiments carried out. Again, adults were of comparable ages.

Other Chymomyzid Species

Single male aggregations have been recorded for a number of insect species, including Hawaiian *Drosophila* (Thornhill & Alcock, 1983). Males of different species share the same lek in Australia (Ehrman & Parsons, 1981; Parsons, 1982, 1983). *Drosophila melanogaster* males have demonstrated lek behavior in Yugoslavia (Taylor & Kekic, 1988). Other *Drosophila* species are attracted to the same site year after year (Carson & Stalker, 1951). Attraction of chymomyzids to fresh cut fire wood was noted in 1985 at MLBS. A chymomyzid species aggregation was observed at a natural site in 1986. Species coming were determined over a two-week period in July. Attraction to old versus fresh damaged trees and observations on species present were extended into August in 1987 until no more were attracted to the site.

RESULTS

Substrate Utilization

A single *C. amoena* pair from an October 1978 EJ apple collection produced 34 F_1 on fresh commercial apples. Mass matings among sequential emergees had an average emergence-to-oviposition interval of 8.7 $\pm$ 1.4 days and produced 81 F_2, again on fresh apples. The four pairs emerging from native crabapples produced 52 adults on unripe apples and 51 adults on ripe apples after being transferred to this substrate. The following year adults presented simultaneously with unripe frassy apples and ripe apples supplied with frass oviposited on both but 76 adults emerged from the unripe frassy apples, 32 adults from the ripe frassy apples ($\chi^{22}_{1} = 17.0$; $P < 0.005$). Data are the pooled results of three replicates which are similar.

Larval Behavior and Pupation Site Choices

In 1985, 46 larvae pupated in soil and 76 pupated in situ among the 122 *C. amoena* adults emerging from 9 native crabapples collected in May. In 1986, 49 migrated to the soil, 127 pupated in situ among the 176 adults from 20 native crabapples. In 1985, 11 larvae pupated in soil and 30 in situ among the five apples from the initial mid-July collections from which *C. amoena* adults emerged. Both *Drosophila* and *C. amoena* adults emerged from the later July 1985 collection (Band, 1988a), complicating determination of behavioral polymorphism for pupation site choice. In 1986, 13 of 16 adults emerging from the initial apple collection migrated to the soil; only three remained with the substrate. Our interest here is not in the effects of the 1986 Southeast drought on numbers surviving but in the persistence of variation for migrating versus remaining with the substrate at the time of puparition. Overall figures for both years indicate that 32% of the Michigan and 42% of the Virginia larvae pupariated in soil.

The 33 walnuts inspected in spring 1985 had 2 pupae on the outside, 27 inside and 3 still larvae after being held overwinter in an unheated shelter; the 12 walnuts yielding a November 1984 population showed 12 pupae on the outside.

Mass migration typically results from disturbing a culture. Plate 1.c shows pupae on tissue following larval migration 24 hours previously.

Natural and Laboratory Populations of C. amoena

Mating pairs of *C. amoena* were not observed at East Jordan, Michigan. Battles between adults, presumably males, were lengthy, wide-ranging, but inflicted no damage. Larger aggregations of adults at site A Lansing seemed not to diminish the intensity of fights between any two individuals, just the scope of the territory covered in chasing. Aggression is present in a variety of insects, including Hawaiian drosophilids (Thornhill & Alcock, 1983). Seven adults, all males, captured at site A in early July 1981 were on immature fallen apples. Kept on them in the laboratory, all died within 24 hours. Five adults (3 females, 2 males) captured the next day on the same substrates were transferred to medium + apple and produced eggs in 3 days. A pair captured in 1982 also produced eggs in 3 days, given commercial apple only.

In 1981, 18 of the 22 immature fallen apples on which flies were displaying (wingwaving) at site A had *C. amoena* eggs. In 1982, 17 of 21 immature fallen apples at site B had *C. amoena* eggs. There is a significant probability ($\chi^2_2 = 15.72$, $P < 0.001$) that a displaying adult will be on an apple on which a *C. amoena* female has already oviposted. In *Drosophila* both sexes can be attracted to sites where gravid females

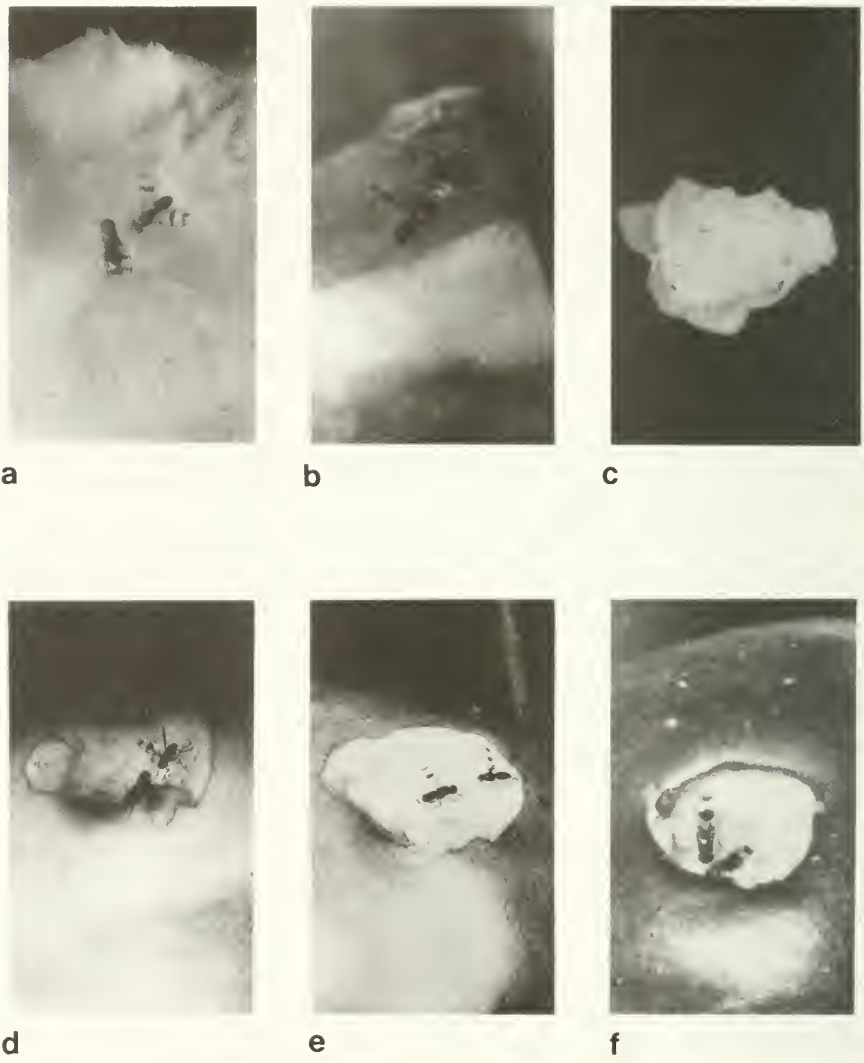


Plate 1. a) A *C. amoena* male approaches a female; b) A female with uplifted abdomen, the male mating avoidance position; c) Pupation in tissue following larval mass migration; d) Forelegs uplifted; in "splaying" both forelegs are extended outward, then sidewise simultaneously as in a swimmer's breaststroke; e) A hostile encounter showing single wing elevation by each individual; the fly on the right is a female; f) Two individuals (two females or a male and a female) may share a feeding site.

have been (males: Spence et al., 1984; females: Mueller, 1985). When adults were placed in population bottles with unripe fallen apples comparable to those on which adults had been captured, all died within two days. However, 31 of the 179 fallen apples collected 13 June 1985 contained internal frass and 7 had 23 *C. amoena* eggs on the exterior.

Laboratory observations reveal that females elevate the abdomen to avoid "courting" males, as shown in Plate 1.b. Females may also rotate their wings to 90° angles to the body in such a position, and continue feeding. Ehrman & Parsons (1981) describe the uplifted abdominal position in other *Drosophila* females but to date this has not been seen in natural populations of *C. amoena.* Females in nature do not approach males until past their prefertile period.

Capture-in-the-air-type matings have been observed among Michigan, Missouri and Virginia populations in laboratory cultures; here a male on the top or side of the population bottle leaps on the departing female and the two glide to the bottom of the population bottle where mating may or may not occur. The assault-type mating system (Wheeler, 1952) is also inefficient as practiced in this species since a male mounted on a female may not be "in copula." Courtship is simple when practiced and consists of tapping the female from any direction. In laboratory culture, males may approach females, as seen in Plate 1.a, or females approach males. A mating pair continues to be mobile, the female carrying the male. Although rape has received some attention among other animal species (Alcock, 1979; Thornhill & Alcock, 1983; Krebs & Davies, 1987), only one possible incidence to date has been noted among laboratory *C. amoena;* this was among the slowest emergees in the October 1978 F_1 cultures where a female fled after mating. Females usually struggle free or dislodge a male by kicking. Similar behavior is described for *Scaptomyza* females (Carson, Hardy, Spieth & Stone, 1970).

Both sexes have the capacity to move sidewise, to wing-wave, to splay the front feet while wing-waving, to pulsate the abdomen by rapidly depressing it downward, to "rush" an opponent. Females are also aggressive but are less pugilistic than males; female-female encounters are marked by much wing-waving and rapidly depressing the abdomen. Plate 1.d shows an individual with uplifted forelegs. Plate 1.e shows a hostile encounter between two, one of which is a female. Plate 1.f shows that individuals, two females or a female and a male, will share a limited feeding site. Photographs also depict attraction to fresh damaged apple.

Time to Oviposition, Laboratory Data

Table 1 shows the comparisons of oviposition rates among intra-and interpopulation matings on medium and on apples. The latter includes both crosses between populations within states and between states. Typically when flies are not aged, mating and oviposition appear to take

TABLE 1
Comparison of emergence (e) to oviposition (o) in days for *Chymomyza amoena* in a variety of crosses

Type of Cross	*Number*	*e to o (MEAN ± SEM)*	*F*
On Medium			
Not aged			
MI	7	6.42 ± 0.75	
MO	2	4.50 ± 0.50	
Mid-South	5	8.40 ± 2.25	$F_{2,11} = 1.07$
MI x MI	8	9.25 ± 0.77	
MI x MO	6	10.20 ± 1.01	
MI x Mid-South	6	7.33 ± 1.02	
VA x VA	4	9.75 ± 3.09	$F_{3,20} = 0.87$
Hybrids			
Walnuts	7	9.75 ± 0.82	$F_{1,10} = 5.96$
Crabapples	4	6.50 ± 0.48	($P < 0.05$)
Aged			
MI x MI, MO	12	3.40 ± 0.29	
On Apples, not aged			
1978 MI	3	8.67 ± 1.45	
1986 MI x VA*	4	5.25 ± 0.25	
1986 VA x VA*	4	7.50 ± 1.50	$F_{2,8} = 2.13$

* on frass

place faster when males and females come from the same culture. Interestingly, laboratory grown flies mated with walnut emergees show an oviposition delay comparable to interpopulation crosses; crabapple emergees are more readily accepted. Aged males and females have the same oviposition time as males and females captured together in nature, 3 days. The 1986 crosses using apples + frass demonstrate that Michigan and Virginia flies readily mate and produce eggs in about the same time as intraMichigan cultures on medium. However the use of frass does not significantly speed laboratory oviposition on apples.

Oviposition Preference, New Versus Used Substrates

Females presented with immature apples, matched for size, laid 87 eggs on the ones that had been "seeded" with two *C. amoena* eggs, 42 on the ones that lacked eggs ($\chi^2_1 = 22.2$; $P < 0.005$). Of the 42, 21 were laid in

frass deposited by a pest larva that broke the surface (Band, 1988a). In August 1987, 12 apple collected in Virginia had a total of 134 eggs of which 29 looked new; 33 collected had no *C. amoena* eggs readily evident. In Michigan, 7 apples collected in August at site B contained 35 eggs of which 16 looked new; again 16 other apples lacked eggs (Band, 1988b). All apples had been damaged. Thus natural population results confirm earlier laboratory data that females are attracted to sites of prior *C. amoena* oviposition.

Egg Aggregation In Nature

Negative binomial k values indicating egg aggregation demonstrated no significant differences between Virginia populations (Band, 1988c) or between Michigan populations on different fruits (Band, 1988b). Virginia values for 1986 were 0.26 ± 0.05 (N = 9) for 1987, 0.18 ± 0.02 (N = 9). Michigan values were 0.24 ± 0.09 for eggs on plums (N = 3) and 0.29 ± 0.12 for eggs on apples (N = 3). The similarity of values between the two states is supported statistically. The average k value for Virginia is 0.22 ± 0.03, and for Michigan is 0.26 ± 0.07.

It is however, possible to compare numbers of apples with zero, one or more than one egg. This is shown in Table 2 for 1987 data. Significant heterogeneity becomes apparent in both Virginia (d.f. = 4; G = 20.75; $P < 0.005$) and Michigan (d.f. = 1; G = 8.41; $P < 0.05$) regarding substrates with one versus more than one egg, although again most substrates collected lacked any eggs.

Individually scattered eggs occur on media surface in the laboratory. For instance, 53% of 60 eggs on medium in a Virginia culture and 79% of 88 eggs in a Michigan culture did not touch another egg. Eggs are also oviposited on the surface and are not buried into the medium. In nature females lay significantly more eggs in holes, scars or breaks in the surface (Band, 1988a).

Aggressive Behaviors

Table 3 shows the average number of encounters, fights and mating attempts for 4 populations from Virginia and Michigan at different ages (0-2 days after emergence and 3-6 days after emergence). Table 4 gives the 3-way comparisons a) between age categories within states and b) between states within age categories. Older populations in both states have an increased frequency of mating attempts. Michigan populations in both age categories are more aggressive than Virginia populations, both in number of fights and number of mating attempts. Two Michigan females etherized and dissected at 2 days were immature despite male mating attempts.

Two large Virginia populations assessed for fights and mating attempts at 2 days past emergence had a mean number of 2.5 ± 0.5 fights

TABLE 2
Number of fruits with zero, one or more than one *C. amoena* egg in 1987

State	*Location*	*Number of fruits*	*Zero*	*One*	*> One*
VA	MLH	269	160	30	47
	Mid-700	200	132	13	55
	Blacksburg	237	160	30	47
MI	plums	216	169	25	22
	apples	158	88	18	52

1 vs > 1: VA: d.f. = 2, G = 15.86, $P < 0.005$; MI: d.f. = 1, G = 9.13, $P < 0.05$

TABLE 3
Comparison of *C. amoena* aggression by state and age, small populations, half-hour observations, four replicates (MEAN ± SEM)

State	*Age*	*Encounters*	*Fights*	*Mating Attempts*
VA	0-2 days	37.2 ± 7.92	0.25 ± 0.25	0.5 ± 0.5
	3-5 days	35.0 ± 8.80	1.5 ± 0.96	4.25 ± 2.36
MI	0-2 days	40.5 ± 4.55	1.25 ± 0.25	4.5 ± 1.19
	3-6 days	23.8 ± 2.29	1.75 ± 0.85	12.0 ± 1.47

and 6.5 ± 2.5 mating attempts. They did not differ significantly from small Michigan populations in the same age categories in these two measures. Only one actual mating was observed during these timed observations.

Small populations given a single apple with one hole cut into it and with three holes nevertheless supported only one male of the three aspirated into the container. Females, as expected, laid eggs in the exposed apple flesh.

Other Chymomyzid Species

In 1985 chymomyzid aggregations appeared on fresh cut (oak) firewood at the Station. Morning and evening assemblages occurred. As the only banded wing species, *C. amoena* could be observed courting interspecifically. Other chymomyzids captured fell into two groups, and contained previously unknown species. A larger species no. 1 was

TABLE 4
ANOVA Statistical comparisons of aggression data by state and age

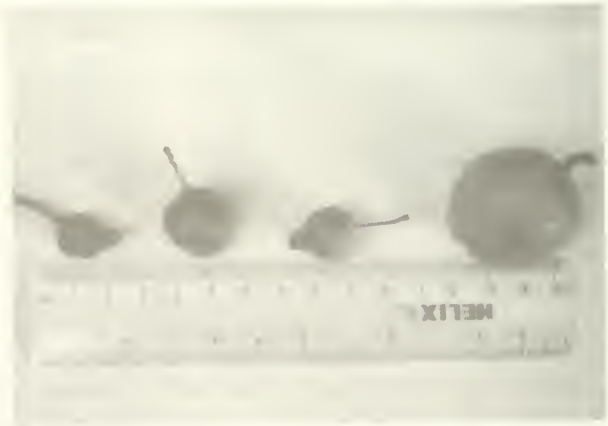
State/Age	*Comparisons*	*d.f.*	*G*	*P*
VA	RxCxA Independence	17	79.45	< 0.005
	AxC Independence	2	17.41	< 0.005
	RxA Independence	3	19.57	< 0.005
	RxC Independence	6	22.63	< 0.005
	RxCxA Interaction	6	19.84	< 0.005
MI	RxCxA Independence	17	54.30	< 0.005
	AxC Independence	2	33.39	< 0.005
	RxA Independence	3	7.93	< 0.025
	RxC Independence	6	7.74	n.s.
	RxCxA Interaction	6	5.25	n.s.
A = age, R = replicates, C = behaviors				
Younger	RxCxA Independence	17	37.24	< 0.005
	AxC Independence	2	14.94	< 0.005
	RxA Independence	3	8.98	< 0.05
	RxC Independence	6	2.75	n.s.
	RxCxA Interaction	6	10.57	n.s.
Older	RxCxA Independence	17	79.47	< 0.005
	AxC Independence	2	23.61	< 0.005
	RxA Independence	3	14.86	< 0.005
	RxC Independence	6	11.04	n.s.
	RxCxA Interaction	6	29.96	n.s.
A = state, R = replicates, C = behaviors				

captured initially, a smaller species later. A pair of large chymomyzids included a species no. 1 male and another chymomyzid, now retrospectively, a *C. caudatula* male from its posterior anatomy (Band, 1986), after the capture of this species in 1986 and a species no. 1 female in 1987.

Species aggregations for 1986 and 1987 are shown in Table 5. Both occurred on damaged trees at elevations comparable to the Station grounds. The 1986 tree was wild cherry *Prunus* sp. (Plate 2.a); the wound measured 12.7 x 38 cm and was ringed by sap but no eggs or larvae were found in samples taken. Chymomyzids were captured between July 15 and July 25. Trapping and identification demonstrate a maximum of four and a minimum of two species were present, as shown in Table 5. Dark and light forms of both *C. procnemoides* and *C. aldrichii* were present. *Chymomyza caudatula* was captured only over the first three days but fighting between unequal sized males demonstrated

a

b



c

Plate 2. a) The 1986 lek chymomyzid tree, a wild cherry *Prunus* sp.; b) The 1987 lek chymomyzid tree, a striped maple *Acer pensylvanicum;* c) Size range of fallen apples used by *C. amoena* females for oviposition in Southwestern Virginia in early July 1987.

TABLE 5
Occurrence of chymomyzid species in Virginia's Allegheny Mountains in July 1986 and July and August 1987.

Species	*Dates*						*July 1986*				
	15	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*
C. caudatula	———										
sp. no. 2			x								
C. procnemoides	———	———	———	———	———	———					
C. aldrichii			———	———	———	———					
C. amoena	x		x								

	July and August 1987														
	18	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*1*	*2*
C. procnemoides	———	———	———	———	———	———	———	———	———	———	———	———	———	———		
C. aldrichii				———	———	———	———	———	———	———	———					
sp. no. 1 female	x															

interspecific aggression. Mating pairs were observed beginning on July 16. Mating interference was also observed. Thornhill and Alcock (1983) argue that mating interference is a cost of male aggregation.

The 1987 tree was a striped maple *Acer pensylvanicum;* the wounds were extensive and there was no evidence of accumulated sap exudate (Plate 2.b). Both *C. procnemoides* and *C. aldrichii* shared the site and a single species no. 1 female was also captured. In these more shaded 1986 and 1987 locations, aggregations could be observed throughout the day. At a lower elevation on a lot being cleared, both *C. procnemoides* and species no. 2 chymomyzids were captured.

In 1986 of the 37 individuals trapped, 76% were males (Band, 1987). In 1987 22 individuals were trapped and 70% were males. The excess males to females at chymomyzid leks is as expected (Watabe, 1985; Band, 1986) and found by Parsons (1982, 1983) at the lek sites of Australian lek *Drosophila*.

A captured *C. aldrichii* male was placed with a *C. aldrichii* female, collected a day earlier, on Stalker's potato medium in the laboratory. Mating was observed the next day. However, both died within four days; the female was dissected and had two eggs ready for oviposition. Again, this supports evidence from *C. amoena* that females in nature only approach males past a lengthy prefertile period.

TABLE 6
Behavioral comparison of preadult traits

Chymomyza	*Also observed in*	*Reference*
Larvae		
cold-hardy	Tephritidae	Storey & Storey (1986)
	Otitidae	Band (1988a)
	Hemiptera, Hymenoptera	Somme (1982)
	Coleoptera, Lepidoptera	Somme (1982)
	Diptera	Somme (1982)
	Drosophila deflexa	Basden (1954)
Puparia		
in situ	Tephritidae	Storey & Storey (1986)
on surface	some drosophilids	Grossfield (1978)
in soil	Tephritidae	Dean & Chapman (1973)
	many Hawaiian *Drosophila*	Grossfield (1978)
Breeding substrate		
firm fruits	many insect groups	
	Lissocephala	Lachaise (1977)
fleshy fruits	*Scaptomyza*	Brncic (1983)
	s-g* *Scaptodrosophila*	Parsons (1983)
	s-g *Sophophora*, *Drosophila*	Parsons (1983)
	Tephritidae, Otitidae	Borror et al. (1981)
fresh fruits	s-g *Scaptodrosophila*	Throckmorton (1975)
	Tephritidae	Borror et al. (1981)
nut husks	Tephritidae	Borror et al. (1981)
acorns	Coleoptera	Borror et al. (1981)
frass	some *Drosophila*	Heed (1968)
wood	many insect groups	

*s-g = subgenus of the genus *Drosophila*

DISCUSSION

Tables 6 and 7 summarize behavioral comparisons of chymomyzids to well studied drosophilid genera, subgenera and other insect groups. Larval traits are surveyed in Table 6, adult traits in Table 7. Chymomyzids display a mixture of drosophila-like, nondrosophila-like and non-drosophilid behaviors. They span preadult, adult, male and female behaviors. Larval overwintering, wing-waving, male aggregations, capture-in-the-air or assault-type mating, and the use of firm substrates or decaying wood are primarily nondrosophila, nondrosophilid or found among the Hawaiian drosophilids. Foreleg splaying (Grimaldi, 1986;

TABLE 7
Behavioral comparison of adult traits

Chymomyza	*Also observed in*	*Reference*
Both sexes		
wing waving	Otitidae	personal observation
	Tephritidae	Borror et al. (1981)
	Hawaiian *Drosophila*	Spieth (1982)
	Drosophila tetraspilota	Grossfield (1978)
Males		
aggressive	Otitidae	Thornhill & Alcock (1983)
	Tephritidae	Thornhill & Alcock (1983)
	Hawaiian *Drosophila*	Spieth (1982)
lek behavior	Otitidae	Thornhill & Alcock (1983)
	Tephritidae	Bush (1975)
	Hawaiian *Drosophila*	Thornhill & Alcock (1983)
shared leks	Australian *Drosophila*	Ehrman & Parsons (1981)
aggregations	Otitidae	Thornhill & Alcock (1983)
	Tephritidae	Thornhill & Alcock (1983)
	Hymenoptera	Thornhill & Alcock (1983)
	Hawaiian *Drosophila*	Spieth (1982)
	Australian *Drosophila*	Parson (1983)
	Drosophila melanogaster	Taylor & Kekic (1988)
assault-type mating	Tephritidae	Thornhill & Alcock (1983)
	Scaptomyza	Carson et al. (1970)
capture-in-air	Hymenoptera	Thornhill & Alcock (1983)
Females		
mating avoidance	s-g *Sophophora*	Ehrman & Parsons (1981)
aggressive	Hymenoptera	Thornhill & Alcock (1983)
	Hawaiian *Drosophila*	Spieth (1975)
eggs on surface	*Scaptomyza*	Grossfield (1978)
eggs singly	s-g *Antopocerus*	Grossfield (1978)
(one per site)	some Hawaiian *Drosophila*	Grossfield (1978)
eggs not clustered	*Scaptomyza*, some	Grossfield (1978)
	Hawaiian *Drosophila*	Grossfield (1978)
eggs clustered	some Hawaiian *Drosophila*	Grossfield (1978)
	s-g *Sophophora*	Grossfield (1978)
aggregation	s-g *Sophophora*	Grossfield (1978)

Band, 1988a) not described among *Drosophila* or other groups, may be unique to *Chymomyza*.

Larval overwintering by *C. amoena* in exposed substrates is both a physiological, and a morphological difference from cosmopolitan drosophilids that can share fruit substrates in summer (Band, 1988a, c).

Among the forest chymomyzids, *C. costata* is known to breed and overwinter in decaying wood (Enomoto, 1981). The fat body is the most conspicuous organ in a chymomyzid larva. Its overdevelopment protects the brain and other internal organs. Possibly all insect species overwintering in the larval stage have overdeveloped fat bodies, suggesting in this case that behavior is strongly correlated with morphology and development, all of which have undergone radical modifications within the insects, especially with regard to nonlarval overwinterers (Somme, 1982; Zachariassen, 1985).

The single most typical adult chymomyzid trait, wingwaving, is also typical of otitids captured displaying on wood near Mt. Lake. Otitids are also aggressive and have male aggregation hormones (Thornhill & Alcock, 1983). One otitid, *Euxesta notata* larvae, overwinters with *C. amoena* larvae in Michigan orchard apples in winter (Band, 1988a); adults wing wave. Tephritids called "peacock flies" also wing wave (Borror et al, 1981). Recently, Spieth (1982) described this behavior among the primitive picture-wing Hawaiian *Drosophila*, the *planitiba* group, which are also the most pugnacious.

Rhagoletis species, which also are tephritids as is the cold hardy larval overwintering gall-forming *Eurosta solidagenis*, display capture-in-the-air, assault-type matings, and are aggressive (Bush, 1975; Thornhill & Alcock, 1983). Citing Prokopy and Heindrich (1979), Thornhill & Alcock (1983) note that Mediterranean fruit fly males may be attracted to fruits used by ovipositing females. Thus the fact that *D. melanogaster* and *D. simulans* males are attracted to sites where gravid females of their species have been (Spence et al., 1984), is also found in *C. amoena* and possibly distantly related tephritids. Gravid females, whether their hormones encourage or discourage others of their species from using the same oviposition site, announce the location of acceptable substances for larval development. The *Scaptomyza* have assault-type mating.

Male aggregations for the purpose of attracting mates, as displayed especially by the forest chymomyzids, is of more limited occurrence (Thornhill & Alcock, 1983, Table 6.2), although reasons for lekking continue to be controversial (Krebs & Davies, 1987). However, tephritids, otitids and hymenopterans are among those having males that track olfactory signals and aggregate. Possible differences among chymomyzids in display postures and prancing have been suggested (Watabe, 1985). Sympatric occurrence of mating pairs of *C. procnemoides* and *C. aldrichii* near MLBS in 1986 (Band, 1987) supports this conclusion. However, size, foreleg color and its extent (Wheeler, 1952) distinguish a majority of the males in the MLBS vicinity. Multiple species' male aggregations occur for relatively brief periods in Virginia's Allegheny Mountains. Chymomyzid adults are attracted only to fresh cut wood or fresh damaged trees.

The relationship of the Hymenoptera to the other insect orders is still debated (see Hennig, 1981). Hymenoptera recognize colony odor, have aggressive females, matings in the air, and react as a colony to disturbance. Some species have queens which overwinter, which enter into burrows or tunnels for protection as well as oviposition (Thornhill & Alcock, 1983). Parasitized apples frequently have to be dissected to demonstrate that *C. amoena* females have oviposited inside.

The use of firm substrates for oviposition probably hindered the discovery that *C. amoena* invasion of apples and other fruits has been widespread (Band, 1988a, b, c). Adult females, however, display the drosophilid need for a damaged surface (Carson and Heed, 1983: Band, 1988a, b). Bract oviposition, practiced by the fig-breeding lissocephalids (Lachaise, 1977) is rare in *C. amoena*. Previously, the fig-breeding African lissocephalids were the only drosophilids known to oviposit in unripe fruits. *Lissocephala* breeding in the nephritic gills of crabs have a high tolerance for a nitrogenous environment (Carson, 1974), similar to that imposed by frass breeding.

Therefore, despite being a drosophilid, *Chymomyza amoena* in particular displays undrosophila-like affinities while the multiple species' male aggregation of the forest group has been reported only for Australian *Drosophila* (Ehrman & Parsons, 1981; Parsons, 1982, 1983). It may be atypical for other species with aggressive males. *C. amoena* males distributed individually on a group of fallen apples may represent a male aggregation, although apples chosen have typically already been used for oviposition.

Comparing chymomyzids to other drosophilid genera and subgenera, again behavior demonstrates multiple affinities. *C. amoena* and *Scaptodrosophila* association with fresh fruits supports Hackman et al. (1970) that these two taxa are related. Unknown at that time was the range of adult and preadult traits shared with the *Scaptomyza* and the lek *Drosophila* in addition to the *Sophophora* as shown in Tables 6 and 7.

Observational data on behavior, emphasized by Ehrman (1978), confirm affinities of the *Chymomyza* to the Sophophoran subgenus but are more in agreement with Throckmorton's (1966) suggested association of the chymomyzids to the *Scaptomyza* and Hawaiian *Drosophila*. Chymomyzids could have diverged early from the Sophophoran lineage or alternately be at or near the drosophilid stem. The latter is suggested by Beverley & Wilson (1984), but anticipated by Throckmorton (1962, 1966).

Small population size and a predominant forest nature of the chymomyzids (Okada, 1981; Bachli and Rocha-Pite, 1981; Grimaldi, 1986 and here) including the wood breeding habitat (Enomoto, 1981; Grimaldi, 1986) again refocuses on the question of the origin of the drosophilids. African high altitudes would have promoted cold hardi-

ness (Somme & Zachariassen, 1981) while retaining an African origin of the chymomyzid stem group (Wheeler, 1963). The feeding habits of *C. procnemis* remain unknown despite its presence in Hawaii and Japan (Wheeler, 1981). Ethanol concentration affects host selection in "secondary" bark beetles (Klimetzek et al., 1986). Nevertheless, this agrees with Throckmorton (1975) that adaptation to alcohol in the environment and exploitation of the fermenting fruit niche (Parsons, 1982, 1983; Mueller, 1985) came later in drosophilid evolution. Molecular studies on the alcohol dehydrogenase polymorphism (Aquadro et al., 1986) and comparative molecular data of *D. simulans* and *D. mauritania* to the *D. melanogaster Adh*^{4} allele (Cohn and Moore, 1988) support this conclusion.

The phenotypic plasticity manifested especially in all stages of *C. amoena* life cycle suggests chymomyzids retain traits from the primitive drosophilid ancestor which have undergone differential evolution in the drosophilid radiation. Certainly behavior gives little support to MacIntyre & Collier's (1986) inclusion of the chymomyzids in the genus *Drosophila* while linking them to otitids and tephritids more strongly than suggested by LSP-2 analysis (Beverley & Wilson, 1982, 1984). It also indicates that parallel evolutionary divergence has occurred in both families regarding behavior associated with feeding, breeding, development and overwintering.

The significant geographic difference between *C. amoena* populations in aggressive behavior indicates that aggression is a trait that has also been subject to divergent selection and parallel evolution both in the Drosophilidae and the superfamily Tephritidae. Although much attention has been focused on males in the evolution and speciation in Hawaiian *Drosophila*, both male and female behaviors have been subject to evolutionary modification in other lines. Wing-waving and aggressive behavior shared by chymomyzids and primitive picture-wing Hawaiian *Drosophila* females reveal unexpected affinities, paralleling the assault-type mating system shared between chymomyzids and scaptomyzids. Behavior therefore argues for a stem position of the chymomyzids to the genus *Drosophila*. Behavioral affinities to the otitids and tephritids, in particular, support an acalypterate origin, as postulated by Borror et al. (1981).

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FEMALE AGGRESSION IN ALBINO ICR MICE: DEVELOPMENT, SOCIAL EXPERIENCE, AND THE EFFECTS OF SELECTIVE BREEDING *(MUS MUSCULUS)*

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ABSTRACT: Social experience has been shown to mask or eliminate heritable effects on aggressive behavior in male mice. This work assesses the impact of social experience in females from lines of mice selectively bred for differential male aggressiveness. These results confirm the earlier report of cross-sex similarity in aggressive behavior after selection directed only at male behaviors (Hood & Cairns, 1988). Repeated test experience increased aggressive behavior of S_0 females. In addition, a genetic-developmental interaction was found, with enhanced aggressiveness in mature vs. young high-aggressive line females. Repeated test experience in 4 daily trials with mature S_{15} females obscured the clear line differences in attack frequency obtained on the first trial. In particular, a few highly aggressive individuals emerged among the group-reared low-aggressive line females. Isolation housing did not alter female aggressiveness. These findings are discussed in regard to conceptions of genetic-experiential-developmental interactions, and the role of female social behavior in microevolutionary processes.

How genetic and experiential factors influencing aggressive behavior are fused in ontogeny has been the focus of recent investigations of mice selectively bred for differential male aggressiveness (Cairns, MacCombie & Hood, 1983). A central concern in this analysis has been the role of contextual and developmental factors in sex and line differentiation. Previous research demonstrates that, when sex-appropriate developmental and contextual assessment conditions are employed, the behavioral phenotype of males and females shows similar responsiveness to selection pressure based only on the behavior of males (Hood & Cairns, 1988). The present research extends those findings to determine whether female line differentiation is maintained after continued selective breeding based on male behavior, and to assess the influence of social experience on the development of line differences in female aggressive behavior.

The developmental impact of social experience in male aggressiveness has been demonstrated in these lines of mice. Isolated male mice

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show heightened aggressiveness at puberty (for example, Cairns, Hood, & Midlam, 1985; Cairns & Nakelski, 1971), and either repeated testing (Cairns, MacCombie, & Hood, 1983) or group rearing (Cairns & Hood, 1983; Hood & Cairns, in press; Lagerspetz & Lagerspetz, 1971) is sufficient to mask or eliminate selective breeding effects on intermale aggressiveness.

The evidence on social experience effects in female aggressiveness derives from research on a variety of lines and strains of mice, and the results are not so consistent. In wild-type mice selectively bred for differential female aggressiveness (Hyde & Sawyer, 1980), isolation-reared females score higher than group-reared females on a variety of social-investigatory measures, and line differences are maintained in both isolation and group-rearing conditions. (Also see Weltman, Sackler, Schwartz, & Owens, 1968). However, studies by Gray (1979, Gray, Whitsett & Ziesenis, 1978) indicate that in ICR female mice, isolation housing decreases aggressive behavior. Two investigations of selective breeding effects in mice failed to show any attacks at all by isolation-reared females (Cairns, MacCombie, & Hood 1983; Lagerspetz & Lagerspetz, 1975).

To clarify the developmental-genetic analysis of female aggressiveness, pilot work was implemented with the S_5 generation of selectively bred ICR mice. Thirteen females from the low-aggressive line and 17 from the high-aggressive line were reared in isolation and tested at maturity, age 200 days, in a dyadic test. Not one of the females attacked their same-age, same-sex test partner. In the S_6 generation, females were reared in small groups and tested longitudinally in the home cage with a same-age, same-sex intruder at seven points in the life-span. In this procedure, females from the high-aggressive line exhibited vigorous and repeated attacks against the intruder, in tests at maturity (Hood & Cairns, 1988). The comparison of these two outcomes suggests that females will attack and will show line differentiation, but only when they are tested at maturity, after being reared in a social context. The implication that female aggressiveness increases at midlife suggests that females show a developmental pattern rather unlike the male pattern of increased aggression at puberty. However, inferences about sex-related differences in the developmental function of aggressive behavior are limited by the experimental design employed in this work. Necessarily, the effects of maturation and the effects of test experience are confounded in the longitudinal design.

The research presented in this article is designed to separate maturation and experience effects by comparing same-age naive and longitudinal groups of selectively-bred females, tested at two points in development (Experiment I), and by testing mature females on 4 successive days (Experiment II). In the short-term longitudinal design of Experiment II, the effects of test experience are independent of age. The ubiquity of genotype-environment interaction is also examined in

Experiment II: the effects of a social rearing context on female aggression are assessed by comparing isolation-reared and group-reared females from the three male-selected lines, high-aggressive, low-aggressive, and control. By testing females from the 6th and the 15th generations of selective breeding, the generality of the previous findings of cross-sex similarity in response to selection (Hood & Cairns, 1988) will be evaluated in advanced generations.

EXPERIMENT I

Effects of Test Experience in Group-Reared Young and Mature Females from Selectively Bred Lines

This work assess the development of female aggressive behavior in three social contexts: one which maximizes social experience with dissimilar conspecifics by housing animals in genetically diverse groups and introducing strange females to the group at intervals; one which offers undisturbed social cohabitation with genetically diverse conspecifics; and one in which genetically similar females co-reside without disturbance. The comparison of genetically diverse and genetically uniform social contexts was designed to reveal genotype-environment interaction in regard to social structure in small groups. For example, we have observed in males that long-term housing with a high-aggressive line male may stimulate uncharacteristically intense aggressive retaliation by low-aggressive line males (Hood & Cairns, in press). Social processes among females may operate in a parallel, opposite, or unrelated manner.

Animals

Females (N = 270) from outbred albino ICR (Institute for Cancer Research) stock in the sixth generation of a selective breeding program at the University of North Carolina at Chapel Hill to establish high-aggressive, low-aggressive, and control lines of mice (Cairns, MacCombie, & Hood, 1983) were studied. In the bidirectional selective breeding procedure, male aggressiveness was assessed in each generation in standard 10-minute dyadic tests at 45 (± 2) days of age, after males had been housed alone since weaning (day 21). Males most likely to attack were mated with sisters of other high-aggressive males to produce the high-aggressive line in each generation, and males with no aggressive behavior were mated with sisters of other nonaggressive males to produce the low-aggressive line. The control line was bred from non-selected animals, derived from the same foundation stock. Line differentiation was rapid and distinct by the S_4 generation. Although female

aggressiveness was not considered in selective breeding, the selection of males produced changes in female aggressiveness that were essentially parallel and equal in magnitude to changes in male aggressiveness, when sex-appropriate test were employed (Hood & Cairns, 1988).

Housing and Rearing. Females were reared in litters of 10 or fewer pups, 5 males and 5 females, culled at day 3 after birth. They were randomly assigned at weaning (day 21) to one of two group-rearing conditions: genetically similar groups with three group members from the same line, or genetically heterogeneous group with three group members from different lines. In each condition, females were housed in standard mouse compartments, 28 x 18 x 13 cm. with two other same-age females, and all were dye-marked for individual identification. 261 females were assigned to 87 groups, 53 same-line groups (20 from the high-aggressive line, 20 from the low aggressive line, 13 from the control line), and 37 different line groups, each group containing one female from each of the three lines.

Test partners were 103 same-age, naive ICR females from unselected stock, reared in groups of 3 to 5 females.

Water and lab chow were continuously available, and all groups were maintained in the same colony room, with a 12:12 reversed light cycle, and constant temperature (22° C ± 2). Cages were changed weekly, except during the week before behavior assessments.

Test Procedures. Groups of females were tested for aggressive behavior in 10-minute intruder trials. During the dark portion of the photoperiod, at least 1 hour after dark onset, the subjects' cage was placed on an observation table in the colony room, under dim red illumination, with water bottle and food removed and wire top in place. After a 3-minute pause, a novel same-age ICR female was placed into the group's home cage. Attacks by each cage resident were coded by an observer, who was blind with regard to the line of the subjects. The coding method used in this series is a continuous time-sampling procedure; attack frequency is the number of 5-second intervals of the 10-minute trial, in which an attack occurred, by a particular animal. Attacks were coded only when a subject forcefully pounced upon a conspecific with biting and wrestling. Other aggressive behaviors, such as bites, feints (lateral display) and lunges (striking with the forepaws) were not included in these scores. Interrater reliability was high (r = .95 to .98). If no attack occurred, the maximum latency score was assigned (600 sec.). After the 10-minute observation period, the intruder was removed, weighed, and placed into a holding case.

Different-line groups were assigned to one of three test schedules. Sixteen groups were tested in a longitudinal series at days 30, 46, 90, 210, 270, and 500 (Hood & Cairns, 1988). Here we report the day 90 results for

16 groups, and day 270 test results for the 13 longitudinal groups that were intact at that age. Ten naive groups were tested at day 90 only, and 11 naive groups were tested at day 270 only.

Same-line groups were tested at day 90 only (26 groups, 10 from the high-aggressive line, 6 from the control line, and 10 from the low-aggressive line), or at day 270 only (27 groups, 10 from the high-aggressive lines, 7 from the control line, and 10 from the low-aggressive line).

RESULTS

The influence of developmental stage and the manipulation of testing and rearing conditions on aggressive expression is conditioned by the genetic background of these female subjects. In a 3 x 3 x 2 (line by test by age) analysis of variance, selective breeding line and age interact in attack frequency ($F(2,294) = 3.67$, $p = .03$) and latency ($F(2,294) = 3.55$, $p = .03$). Selective breeding line interacts with the factor of test condition for attack frequency ($F(4,294) = 2.34$, $p = .05$) and for latency ($F(4,294) = 3.38$, $p = .01$). Main effects of line and test condition are significant for frequency ($F(2,294) = 7.14$, $p < .001$ for line; $F(2,294) = 12.46$, $p = .0001$ for test) and for latency ($F(2,294) = 11.68$, $p < .001$ for line; $F(2,294) = 15.05$, $p < .001$ for test). The main effect of age is significant for attack frequency ($F(1,294) = 3.70$, $p = .05$), and not for latency ($F(1,294) = 2.74$, $p = .09$). The three-way interaction is not significant ($p < .25$).

In order to specify the ways in which line interacts with test condition and with age, two sets of post hoc pair-wise comparisons were made. For the line-by-test interaction, there was no prediction of direction of effects between same-line and different-line naive groups. Accordingly, Tukey's (HSD) method of comparing means was applied (Table 1): in every comparison but one, the high-aggressive line females are different from the other two lines, which are not different from each other. The exception is in naive different-line groups: line differences in frequency are not significant, although latency scores are.

The effect of test conditions distinguishes the longitudinal groups from the two naive groups, which are not different: this holds for each of the three lines, in frequency scores. In comparisons of latency scores, test conditions do not change control line scores, but for high- and low-aggressive line females, each of the three test conditions is different from the other two.

Does aggressive behavior change during development? Yes, but only for the high-aggressive line females. To test the hypothesis that female aggressiveness is increased at mid-life (Day 270) relative to the post-pubertal period (Day 90), Newman-Keuls method of comparing ordered

TABLE 1
Aggressive Behavior by S_6 Female Mice Reared with Same-Line or Different-Line Social Partners

Line	*Day 90*		*N Attacking* / *N Total* (%)	*Day 270*		*N Attacking* / *N Total* (%)
	Attack Frequency	*Attack Latency*		*Attack Frequency*	*Attack Latency*	
High Aggressive Line						
Same-line	0.27	591.67	1/30 (3%)	1.70	540.00	5/30 (17%)
Different-line (naive)	0.40	549.50	1/10 (10%)	0.91	493.64	2/11 (18%)
Different-line (longitudinal)	2.73	457.81	4/16 (25%)	7.92	307.69	7/13 (54%)
Control Line						
Same-line	0	600	0/18	0	600	0/21
Different-line (naive)	0	600	0/10	0	600	0/11
Different-line (longitudinal)	0.93	577.81	2/16 (12%)	2.08	556.54	1/13 (8%)
Low Aggressive Line						
Same-line	0	600	0/30	0	600	0/30
Different-line (naive)	0.30	554.00	0/10 (10%)	0	600	0/11
Different-line (longitudinal)	1.67	530.00	2/16 (12%)	0.50	549.58	2/13 (15%)

Note: Different-line groups each contain one female from each line. Longitudinal groups at Day 90 and Day 270 are the same groups, retested.

means was employed. High-aggressive-line females show increased aggressiveness at maturity, and females from the control- and low-aggressive line do not change over age. Comparisons of line differences at each age show the high-aggressive line to be different from the two other lines, which are not different from each other. The exception is in the comparisons of Day 90 females: high-line groups are different from control line, but not different from low-line groups, for frequency of attack.

In summary, the effects of breeding for differential male aggressiveness were found to alter female aggressive behavior in patterns that

reflect both genetic/developmental interdependencies (the line-by-age interaction), and genetic/experiential interdependencies (the line-by-test interaction). In each case, effects are in the predicted directions, with high-aggressive line females, older females, and previously tested females showing enhanced aggressive behavior.

There remains one confounding factor in these results: the day 90 and 270 assessments differ both in the number of trials administered to the longitudinal groups (3 vs. 5) and in the developmental stage of subjects at the time of comparison. Longitudinal subjects in the younger groups may have been too immature to fight, or alternatively, subjects in the older groups may have learned to fight in their 2 extra trials. To clarify the relative contribution of experiential and developmental factors, females in the S_{15} generation were repeatedly tested at age 200 days, in a short-term longitudinal design utilizing massed, or daily trials.

EXPERIMENT II

Effects of Test Experience in Group- and Isolation-Reared Females from Selectively Bred Lines

An alternative approach to longitudinal designs for understanding the influence of social experience is to eliminate contact with conspecifics by rearing animals in isolation. Pilot work with females of these lines suggests that isolation housing will abolish aggressiveness, although the same procedure augments aggressiveness in males from the high-aggressive line (Hood & Cairns, in press). This extreme manipulation produces a genotype-environment interaction in selectively bred males. The inconsistent outcomes of previous studies of isolation vs. group rearing effects on males aggressiveness may reflect unspecified genotype-environment interactions of the different species, strains, or lines of the subjects employed (Hood & Cairns, in press; also see Henderson, 1970; Lagerspetz & Lagerspetz, 1971; Levin, Vandenbergh, & Cole, 1974; Siegfried, Alleva, Oliverio, & Puglisi-Allegra, 1981; Valzelli, Bernasconi, & Gomba, 1974; in females, Scott, Bradt, & Collins, 1986). In another case, some species of mice show female aggressiveness to be less evident than male (Ebert, 1976), while female aggressiveness is equal to or greater than male aggressiveness in other species (Ayer & Whitsett, 1980; McCarty & Southwick, 1979). Similarly, the increase in aggressive behavior over repeated trials observed in isolation-reared male mice (Brain & Poole, 1974), but not in group-reared males (Goldsmith, Brain, & Benton, 1976; Svare & Leshner, 1973), may be genotype dependent (Bannerjee, 1971; Cairns, MacCombie, & Hood, 1983). This study applies massed repeated trials to isolation- and group-reared females, to

identify the components of social experience that may influence fighting in females.

Animals

Females, approximately 200 days old, (N = 130) from the 15th generation of selectively bred ICR mice (Cairns, MacCombie, & Hood, 1983) were studied. In the 11th generation, a parallel colony was established at The Pennsylvania State University from the NC lines. Subjects for this research were females from the 15th generation, bred at Penn State. The conditions for selection, rearing, testing, and coding have remained reasonably constant over the many generations of these selection experiments.

The test partners were 95 same-age females from a non-Swiss albino stock, retired breeders purchased from Harlan Sprague-Dawley, one month before the beginning of the test series.

Housing and Rearing. Subjects were reared in litters of 6-10 animals and weaned at 21 days, at which time females were randomly assigned to either isolation or group rearing. Isolated animals were housed singly in a standard opaque mouse compartment, 28 x 18 x 13 cm. The compartments were kept beside each other on several layers of a laboratory rack, exposing them to airborne odors and noises of the laboratory colony, though no physical contact with other members of their species was permitted following weaning. Thirty-one animals, 13 from the high-aggressive line, and 9 from each of the other lines, were assigned to isolation rearing.

The group rearing condition involved the placement of siblings of the same age and sex into a standard mouse cage, allowing for continuous conspecific contact and interaction. A group consisted of two to five females from the same line. Variation in group sizes reflects losses due to deaths with no additions to a group. Nine groups were formed from each of the three lines.

All animals were marked for individual identification with black dye (Clairol brand) three days prior to testing. Isolation and group rearing cages were maintained in the same colony room, and test partners were housed in the same colony room for six days prior to testing. The test partners were housed in small groups (3-5) in standard mouse cages in the colony room. Otherwise, conditions were identical to those in Experiment I.

Test Procedures. Group-reared and isolation-reared females from each of the three lines were tested daily on four successive days. All testing was conducted in the colony room during the dark portion of the photoperiod. In each 10-minute intruder trial the isolate or group-

reared subjects remained in their home cage, and the female intruder was placed into the subject's cage. Individual intruders were not used more than one time on any test day, and test partners were assigned to different resident groups on each test day. Behavior coding was carried out by the method described above (Experiment I). Again, interrater reliability was high (r = .97 for frequency; r = .98 for latency to first attack).

The analytic strategy follows the recommendation of Hertzog and Rovine (1985) for analysis of variance with repeated measures. In all of the analyses presented below, heterogeneity of variance is acceptable (Huynh-Feldt's Epsilon $>$.75), and mixed-model analyses were reported.

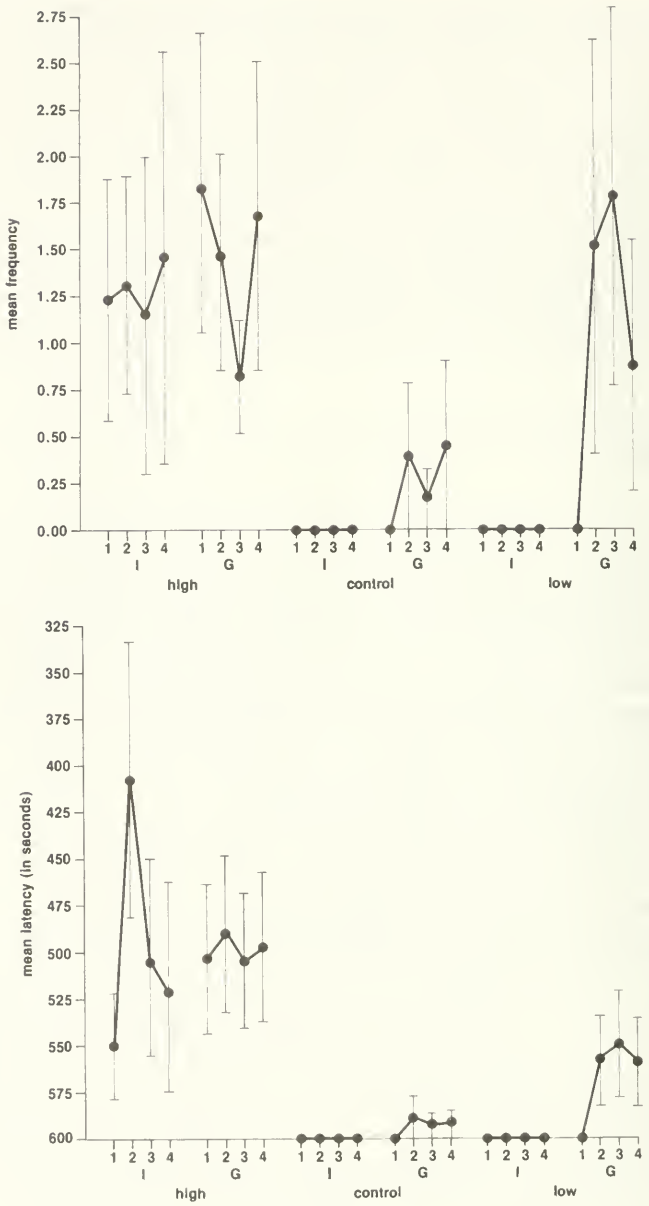
RESULTS

In a 3 x 2 (line by group) repeated-measures analysis of variance including 4 trials as the repeated factor, line differences were robust for latency to first attack ($F(2,124) = 10.99, p < .0001$). Aggressive behavior by females from lines of mice selectively bred for differential male aggression showed clear line differentiation, with high-aggressive-line females attacking fastest at each test occation (Figures 1a & 1b).

All other factors in the global analysis were not significant: the effect of line on attack frequency ($F(2,124) = 2.25, p = .11$), the effect of group vs. isolation housing (for frequency, $F(1,124) = 0.92, p = .34$), the effect of repeated trials (for latency, $F(3,372) = 1.53, p = .21$), and all interactions.

An alternative to the global F-test for assessing experience effects is to compare the results of independent analyses of naive groups (Trial 1) and experienced groups (here, Trials 2-4). This procedure is conservative in that it does not utilize the more precise error term generated by a repeated-measures analysis. Considering each trial independently, frequency scores showed significant line differences on the first trial ($F(2,124) = 5.11, p < .01$) with high-aggressive females attacking most: line differences were not significant on the second, third, and fourth trials. After the first trial, the increased attacks by a few group-reared low-aggressive line animals were sufficient to obliterate line differences. Latency scores showed significant line effects on each occasion.

An additional method of analysis yielded paralled results. In nonparametric analyses of variance of ranked scores on each trial separately, the effect of line is significant at each occasion (χ^2's = 20.79, 13.34, 10.46, 7.81, p's = .001 to .02 for frequency; χ^2's = 20.79, 14.62, 11.71, 8.60, p's = .0001 to .01 for latency). However, inspection of the mean scores in Figure 1 suggests that the control line differs from the high- and low-aggressive lines, after the first trial. The effect of isolation vs. group housing is not significant on any trial.



Figures 1a & 1b. Attack frequency and latency — MEAN and SEM — by females from three lines of mice selectively bred for high levels of male aggressiveness, low levels of male aggressiveness, and a control line. In each line, females were reared in isolation (I) or in small groups (G), and tested on four successive days with a same age (about 200 days) female intruder placed into the home cage.

TABLE 2
Aggressive Behavior by S_{15} Female Mice:
Effects of Isolation vs. Group Rearing and Repeated Test Experience

	N Attacking [a] / % / *N Total*	*N Attacking* *Maximum frequency* *Minimum latency (sec)* [b]			
		Trial 1	*Trial 2*	*Trial 3*	*Trial 4*
High-Aggressive Line					
Isolate Reared	7/13 (54%)	4	5	3	2
		8	6	11	14
		285	5	5	25
Group Reared	13/28 (46%)	5	6	7	6
		16	12	5	15
		20	10	30	10
Control Line					
Isolate Reared	0/9	0	0	0	0
Group Reared	1/38 (3%)	0	1	1	1
		—	15	6	17
		—	185	360	330
Low Aggressive Line					
Isolate Reared	0/9	0	0	0	0
Group Reared	5/33 (15%)	0	3	3	2
		—	34	23	22
		—	15	20	70

a. The number of animals that attacked on at least 1 of 4 trials.
b. Maximum latency score is 600 sec. Minimum frequency score is 0.

A key to understanding this pattern of findings is the wide range of individual differences in attacks over trials (Table 2). Among the group-reared low-aggressive line females, a few highly aggressive individuals emerged after the initial trial, and attacked at extremely high frequencies: in comparisons that include only scores from animals that attacked at least once in the four trials, the trial-by-line interaction was significant (F (3,51) = 3.34, p = .03), with low-line females *more* aggressive than high-line females in the third trial ($F(1,17) = 6.99, p = .02$). This increase in low-line female aggressiveness after repeated test experience offers a comparison to the increased aggressiveness of low-aggressive line males in life-span longitudinal tests (Cairns, MacCombie, & Hood, 1983). These convergent outcomes in females and males underscore the potential influence of repeated test experience in unleashing aggressive behavior by some low-aggressive line animals.

DISCUSSION

Three questions are addressed by the research reported here, and one is clearly settled: line differences in female aggressiveness persist after 15 generations of selection for male behavior. Latency scores are more powerful than frequency scores in discriminating among females from the selected lines, in both generations. Intensification of line differences in female aggressive behavior by the intervening 9 generations of male selection (S_6 vs. S_{15}) is not evident by these measures. (The same-line group scores in Table 1 are comparable to scores in Figure 1, Trial 1 only).

Social experience effects were investigated in three aspects of the two experiments: in comparisons of longitudinal vs. naive groups (Experiment I), in comparisons of social- vs. isolation-reared females and in comparisons of behaviors during repeated trials administered in a daily testing regimen (Experiment I). Longitudinal test experience spaced over the life span augments the expression of aggression in all three selectively-bred lines in Experiment I. The effects of massed test experience in Experiment II were not as clear: among mature S_{15} females, increased aggression over 4 daily trials was found for low-aggressive line animals, when each occasion was analyzed separately. However, these changes were not reflected in the simultaneous analysis of all effects. Similarly, the effect of social vs. isolation rearing is nonsignificant in the global test, but the fact that only group-housed females showed increased aggressiveness over massed trials suggests that isolation rearing may alter female social reactivity in some lines of mice.

Why are the effects of repeated test experience significant in Experiment I, but not in Experiment II? Three possible explanations arise from the differences of design in these two investigations. It may be that massed trials simply are not comparable to spaced trials, as employed in the life-span longitudinal design of Experiment I. Alternatively, the use of subjects that are under continued selection pressure in each generation (S_6 vs. S_{15}) affords the possibility that the phenotypic range of reaction has been shifted. Even after the direct effects of selection on aggressiveness are at asymptote, changes due to selection pressure may yet continue in correlated behavioral systems, as demonstrated by Gariépy, Hood, & Cairns (1988). Finally, the use of ICR strain test partners, each for one test only (Experiment I) vs. non-Swiss albino strain test partners, each tested repeatedly, (Experiment II) may be crucial in interpreting the different outcomes (Hood & Batcheller, in preparation). Attacks by intruders were never observed, but other behaviors or odors may have changed over repeated trials.

The interdependence of genetic-developmental factors, such as sex and line, with experiential factors, such as exposure to social stimula-

tion, may be of general significance for other species. (For a discussion of similar genotype-environment interactions in primates, see Sackett, 1982). The social dynamic that emerges from laboratory and field studies of mice is one in which aggressive adult females are primary agents in the dispersal of juveniles (Ayer & Whitsett, 1980; Fordham, 1971; Healey, 1967; Sadlier, 1965). For example, Savidge (1974) found that in the field, the dispersal of young mice is related to the level of aggressiveness of individual adult females. This regulatory social process may be influenced by familial factors, as demonstrated by studies of live-trapped wild mice (Fairborn, 1978) and voles (Hilborn, 1975). Recruitment of female outsiders into mouse demes may be restricted by the selective aggression of colony females (Chovnick, Yasukawa, Monder, & Christian, 1987; Haug, Spetz, Ouss-Schlegel, Benton, & Brain, 1986; Yasukawa, Monder, Leff, & Christian, 1985). The exclusion of strange females protects colony females from pregnancy block, which can be induced by strange females as well as by strange males (Yamazaki, Beauchamp, Wysocki, Bard, Thomas, & Boyse, 1983), and protects colony young from infanticide (in particular, see the field studies of ground squirrels by Sherman, 1980).

Two themes from the research presented here are in harmony with this view of female roles and social structure: female mice do attack same-sex intruders (also see Hood, 1984), and females from families with highly aggressive males are most likely to fight. This suggests that familial patterns of aggression may be influenced by selection pressures directed at either sex, in interaction with specific and predictable social experiences.

Two additional findings from this research point to characteristics of female aggressiveness that appear to be distinct from male patterns. The ontogenetic pattern of aggressiveness in females shows a peak at maturity, whereas in males there is a sharp onset of aggressiveness earlier in ontogeny, at puberty (Cairns, Hood, & Midlam, 1985; Hood & Cairns, 1988). In addition, the effects of isolation versus social rearing are modest or nonexistent in females of these lines, but quite pronounced in males (Hood & Cairns, in press). However, the exceptions to this conclusion are notable: the few low-aggressive line females that do fight after the initial test experience are markedly mean, and not one of them is isolation-reared. To the extent that there is sex-differentiation of aggressive patterns, fighting among females may serve a sex-differentiated social function in rodent societies (also see Benton & Brain, 1979). If the effect of social experience with intruders is to produce a few effective female fighters in an otherwise pacific group, then, at a population level, gene flow among demes may be modulated in part by interfemale aggressive behavior in response to periodic emigration pressure. Field studies coordinated with laboratory investigations (Schneirla, 1950) will be most useful in refining and testing these hypotheses.

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LEARNING DURING EXPLORATION: THE ROLE OF BEHAVIORAL TOPOGRAPHY DURING EXPLORATION IN DETERMINING SUBSEQUENT ADAPTIVE BEHAVIOR

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ABSTRACT: Two investigations examine the hypothesis that one function of exploration is to create situations in which there is an opportunity to acquire useful information. In the first, male rats (*Rattus norvegicus*, Sprague-Dawley strain) with enriched (EC) or impoverished (IC) experience (leading to differences in exploratory behavior documented previously) were given an opportunity to explore an arena with a hidden escape route on two consecutive days. On the following day, subjects were chased by a mechanical device and the time required to escape the arena was recorded. No group differences were seen in pre-chase behaviors other than those related to the hidden escape route, or in stress-related behaviors while being chased. EC rats escaped significantly more quickly than IC rats, and a composite score derived from pre-challenge behavior in the arena was correlated significantly with escape time under challenge. In the second experiment, EC and IC subjects were chased without previous experience in the area; EC rats escaped significantly more quickly than IC rats. In an analysis of the combined results from the two experiments, both environmental history and pre-challenge arena experience were found to exert significant influence on escape time. These findings demonstrate that different behaviors *during* exploration can lead to functionally significant differences in the information acquired *as a result* of exploration.

Much of the experimental investigation of learning hinges upon studies involving animals under the constraint of having to perform a task which has been imposed by the investigator. Most of animal learning outside of the laboratory, however, is necessarily the product of events that occur during spontaneous activity. Exploratory behavior seems likely as one of the behavioral phenomena through which such learning can take place.

Spontaneous exploratory behavior, outside the context of foraging (e.g., in satiated animals), has been a subject of interest for many years. Both Small (1899) and Slonaker (1912) mentioned behaviors that were apparently inquisitive, and more recently several others have investi-

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gated and offered explanations for exploration (e.g., Berlyne, 1950 et seq.; Glanzer, 1958; Welker, 1961). The typical operational definition, however, has equated spatial locomotion with exploratory behavior (for an exception, see Glickman and Sroges, 1966); measures of locomotion in some variant of the open field test are the primary behavioral variable used in most studies. Although it is widely used, the open field situation has serious defects as a data-gathering technique for the measurement of voluntary behavior. Primary among these defects is the substantial confusion over exactly what is being measured in this test. At the very least, there can be little doubt that behavior in the typical open field test is determined by multiple factors. Considerable evidence exists that measures of locomotion may in fact be measures of the subject's attempt to escape the open field (see, for example, Suarez & Gallup, 1981 and Welker, 1957; the report of Hayes, 1961, occasionally cited as a rejoinder to Welker, 1957, seems not to address the same issues). If an animal is attempting to escape the open field, this implies a motivational state not consistent with behavior patterns under unstressed conditions; it is difficult to attribute cognitive significance to locomotor behavior in such a situation.

In addition to the methodological inadequacy of the open-field test, moving about in empty space is not equivalent to situations most likely to be encountered by animals outside the laboratory. Animals engage in a variety of behaviors while exploring, and many of these behaviors are involved with investigation of specific features of the environment, e.g., objects. Less, however, is known about the nature of object investigation during exploration.

It has previously been shown that exploratory behavior in the laboratory rat *(Rattus norvegicus)* shows experience-dependent changes and that spatial locomotion and object interaction are separable entities. Renner and Rosenzweig (1986) found that adolescent rats with a relatively enriched experience history (designated EC, for enriched condition) showed higher levels of behavioral complexity during interaction with objects than littermates with a relatively impoverished experience history (designated IC, for impoverished condition). These changes occurred without concurrent changes in locomotion or amount of object contact. In adults, changes in exploration appear in both behavioral complexity of object investigation and on several measures of overall quantity of exploration (Renner, 1987).

Although few straightforward empirical investigations have been reported concerning the function of exploration, many hypotheses have been advanced. Welker (1961) states that "learning invariably occurs in any situation that evokes exploration" (p. 201). Some type of hypothesis that exploration might be a part of animal information-processing has

been a common theme, offered also by Glickman and Sroges (1966) and more recently by Toates (1983). The data upon which these speculations are based are, however, often incomplete or indirect.

There is evidence for learning during spontaneous activity, but closer scrutiny reveals it to be learning only about spatial locations, and this learning depends only minimally on the subject's behavior. This evidence dates back to the latent learning studies of Blodgett (1929) and Tolman and Honzik (1930), wherein subjects formed cognitive maps of spatial arrangements without the incentive of experimentally-provided reward. Albert & Mah (1972) extended these findings by establishing that animals can subsequently show memory for the location of an already-relevant stimulus discovered through locomotor activity. These are, however, essentially questions of spatial learning. Furthermore, procedures in these studies were designed so that even random locomotion would have exposed the subject to the information to be learned. Exploration, if it is viewed as functionally meaningful, cannot reasonably be treated as the animal equivalent of Brownian motion.

The purpose of the studies reported in this paper was to find out whether behavioral topography determines what is learned during exploration. Specifically, this investigation is designed to examine the proposition that the specific behaviors displayed during exploration, as well as their organization, contribute to determining the type of information obtained as a result of exploring, and to present evidence relevant to that hypothesis.

In a previous study (Renner, 1987), adult rats with a relatively enriched experiential history showed a different behavioral organization than their experientially impoverished littermates. In this experiment, the clearest environmentally-induced difference in the specific behaviors of adult rats was in the category of climbing: adult rats previously housed in enriched conditions climbed more on large objects than littermates previously housed in impoverished conditions. As a consequence of this behavioral difference between rats with enriched and impoverished experience, there may be differences in the information available to the subjects with different histories; i.e., it is possible that these experientially-induced differences in climbing behavior could in some circumstances lead to changes in the amount and/or type of information acquired about the environment during exploration. The resulting difference in knowledge of the environment could, in turn, be significant under conditions of environmental challenge (e.g., predation). This may be a useful model for study of possible benefits of information acquisition via exploration. This report concerns two studies of the consequences of an environmentally-induced alteration in exploratory behavior for rats' ability to behave adaptively under the challenge of simulated predation.

EXPERIMENT I
Method

Animals

Sixteen male Sprague-Dawley rats, group-housed in standard colony conditions until the beginning of the experiment, were studied. At 90 days of age, the rats were divided into weight-matched pairs and were placed into either enriched (EC) or impoverished (IC) conditions.

Environmental Conditions

Environmental conditions were identical to those described fully by Bennett and Rosenzweig (1981), and so will be described only briefly here. The enriched condition consisted of housing in a group of 12 (including additional filler rats to complete the group) in a large cage (75 x 75 x 40 cm) containing various stimulus objects. Objects were selected from a collection of items kept in the laboratory, including tunnels, wooden playthings, metal enclosures, and junk objects; approximately half of these objects were replaced daily with others from the collection. This procedure provided daily opportunities for investigation of novel objects and novel arrangements of both familiar and unfamiliar objects. In the impoverished condition (IC) each animal was housed singly in a small hanging cage with solid stainless steel sides and mesh floor and front panel. All rats had access to food and water a libitum. Rats in both groups were handled equally and were housed in the same room as the experimental apparatus was located. The room was lighted from 0700-1900 daily.

Apparatus

Tests were carried out in a wooden arena with an available area 120 cm square, surrounded by Plexiglas walls 60 cm high. A round hole 10 cm in diameter was cut in the center of the arena floor, leading to a plastic tub cage (with a layer of wood shavings on its bottom) on a shelf suspended from the underside of the arena floor. The wooden surfaces of the arena were painted medium gray. Black paint lines 1 cm wide divided the floor of the arena into a grid of nine equal-area zones. A wooden box (30 x 40 x 13 cm high) was placed in the center of the arena over the escape hole. A 10 cm hole was centered in the top of the box, and a piece of corrugated cardboard was wedged in the interior of the box to provide a ramp to the arena floor inside the box. This ramp divided the 30 cm drop from the top of the box to the bottom of the tub cage into three steps. The height of this obstruction box had been determined through pilot testing, ensuring that all subjects were capable of easily climbing an

object of this size. A radio-controlled mechanical model car (Nikko RDC 24120, slightly larger than the rats) was used for the simulation of environmental challenge by predation. The model emitted a high-pitched motor noise while it was in motion.

The arena was illuminated by two 25w red bulbs, which were clamped at opposite corners on top of the Plexiglas wall. Dim general room illumination was provided by a single 25w white bulb in a metal shade oriented toward a wall of the testing room, approximately 2 m from the arena; the lamp was positioned so that there was a gap of approximately 3 cm between the metal shade and the wall.

All procedures were recorded on videotape. A low-light video camera was placed in the room for observation of the arena. All additional equipment was in an adjacent room, in order to eliminate the possibility that equipment noise could affect the rats' behavior. The signal from the video camera was passed through a character generator (Panasonic WJ810), which superimposed the current time and date, as well as elapsed time within each session, on a selected portion of the video image. This composite signal was routed to a videotape recorder (Panasonic NV8950). Additional details of the videotaping procedure may be found in Renner & Rosenzweig (1986).

Procedure

After 30 days of differential housing, subjects were moved to holding cages labelled with code numbers such that the tester was blind as to previous housing condition. All testing was carried out under dim red illumination during the first few hours of real-time evening (which was early in the dark phase of the subjects' daily light-dark cycle). The cage rack holding all animals was wheeled to an adjoining room for the duration of each test session to control for possible intrusive effects from olfactory or auditory stimuli. On each of two consecutive nights, each rat's individual cage was carried into the test area and the subject was placed in the arena for 10 minutes. On the first two nights, two novel stimulus objects (chosen from a pool of junk objects kept in the laboratory) were placed in the arena along with the obstruction box described above. The same objects in the same locations were used for a particular subject on both nights. Objects were replaced approximately every fifth subject. Object location was also varied nonsystematically across subjects. Although urine and feces were removed from the arena between tests, the arena was not cleaned with any solvent during the experiment.

On the third night, under lighting conditions identical to those of the first two nights, subjects were individually tested in the arena. Stimulus objects other than the obstruction box were removed from the arena floor prior to introduction of the subject, and a fiberboard box with

paper end curtains was centered against one wall of the arena. After the subject had spent three minutes in the arena a simulated predator (the radio-controlled car described above) emerged from this box; the car was controlled by the experimenter from the next room to chase the subject (without making contact) for 180 seconds or until the subject escaped into the obstruction box.

Although the remote operator attempted to make the model approach the subject rapidly without making contact, this intent was often thwarted by the somewhat erratic nature of the rat's movements under these conditions. As a result, some of the subjects came into infrequent contact with the model.

Behavior in the arena was transcribed from videotape (by observers not familiar with group assignments of individual subjects). Tapes were viewed multiple times to record subjects' location and locomotion, general behaviors not directed towards objects, (e.g., grooming), and investigation of the obstruction box. Day 1, in particular, was examined in some detail, to allow consideration of the possibility that the EC and IC rats reacted differently to the experience of being placed in this situation. Subjects' activity relative to the obstruction box was scored with respect to occurrences and durations for three behaviors that related to the obstruction box: leaning on the box (defined as placing one or both forepaws on the box and interrupting an imaginary plane, formed by extending the vertical walls upward, with the head); climbing onto the box (placing at least three paws in the top of the box); and entering the box (placing three paws inside the box). A summary measure (called a *box score*) was calculated as follows: leaning = 1, climbing = 2, and entering = 3. The day's box score was the value of the highest-scoring behavior exhibited, resulting in the subject receiving a score of 0-3 for each night. A total box score was determined by taking the sum of three nights' scores, including the 10 min sessions on Days 1 and 2 and the initial three minutes of Day 3 (prior to the emergence of the simulated predator).

RESULTS

Behavior in the arena on day 1 was examined in some detail, to allow consideration of the possibility that the EC and IC rats responded differently to the experience of being placed in the arena situation. This study would have been confounded if the behaviors of subjects in the arena had provided evidence that the EC and IC groups had been differentially stressed by the procedure. No such evidence was found. Behaviors relevant to predicting escape did, however, show EC-IC differences.

Group means and the results of statistical tests for several pre-challenge behavioral measures are reported in Table 1. On Day 1, no differences were found in locomotion (as measured by number of zone changes in the arena), or in time spent rearing or grooming. EC subjects spent significantly more time than IC investigating the obstruction box, and exhibited more involved interactions with the box (shown as "box score" in Table 1).

TABLE 1
Pre-Challenge Behaviors

	IC		*EC*			
	mean	*SEM*	*mean*	*SEM*	*F(1,14)*	*P*
Day 1						
Zone changes	86.75	12.73	104.88	5.81	1.467	ns
Rearing (sec)	10.50	1.41	7.00	1.90	1.916	ns
Grooming (sec)	21.13	9.18	38.50	4.92	0.438	ns
Contact (sec)	19.00	7.00	87.75	18.40	10.669	$< .01$
Box score	0.75	0.15	2.13	0.21	24.200	$< .001$
Day 2						
Zone changes	95.87	12.43	76.88	7.07	1.545	ns
Box score	0.75	0.15	1.88	0.32	8.463	$< .05$
Contact (sec)	30.50	7.74	80.875	23.03	3.761	ns
Day 3 (3 min only)						
Zone changes	28.25	5.24	23.13	3.49	0.580	ns
Box score	1.13	0.328	1.75	0.385	1.336	ns

TABLE 1. Pre-challenge behaviors: Day 1 (10 minutes), Day 2 (10 minutes), and Day 3 (3 minutes prior to emergence of radio controlled model predator). Measures relative to the obstruction box were "Contact" (time in contact) and "Box score" (mean level of investigation of the box, as explained in the text).

There were no significant differences between the groups for locomotion and for time of contact with the box on Day 2 (see Table 1). Examination of types of interaction with the box on Day 2 again showed EC subjects as significantly more likely to climb and enter the box than IC subjects. On Day 3, pre-challenge locomotion (within the first 3 minutes of the session) did not differentiate between EC and IC subjects, and level of involvement with the obstruction box during this period was not significantly different between EC and IC groups.

A profile of behaviors under challenge (after the emergence of the simulated predator), adjusted to reflect proportion of pre-escape time rather than actual elapsed time to compensate for different amounts of time spent under challenge, is shown in Figure 1. Measures of these behaviors were quite variable; Analysis of Variance tests of most comparisons were nonsignificant. Relative amounts of time spent in investigation (sniffing at arena walls and floor and the simulated

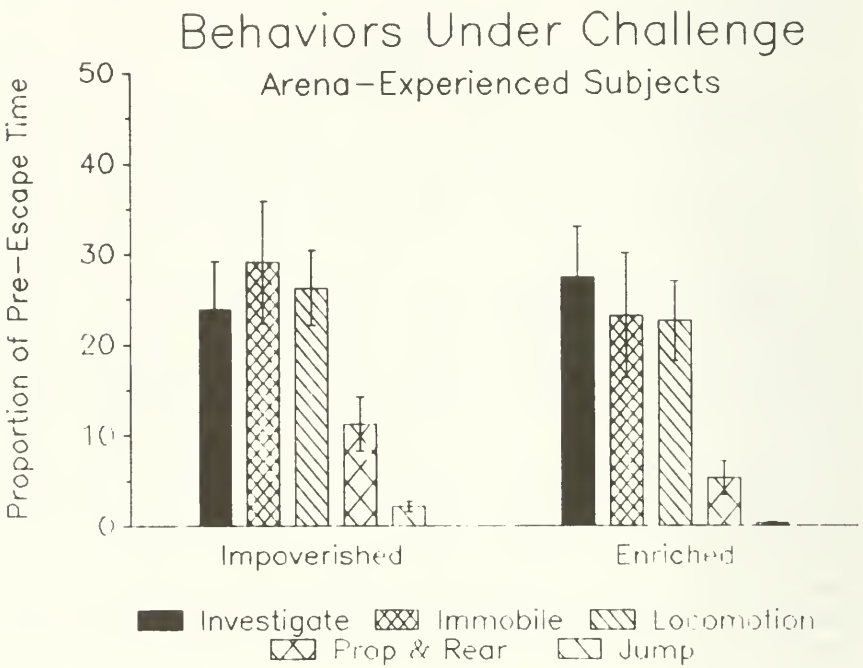

FIGURE 1. Behaviors under challenge, arena-experienced subjects. Bars are mean proportion of pre-escape time (or 180 second session) engaged in these behaviors; bars indicate SEM for each measure. IC subjects were more likely to jump ($p < .005$); all other comparisons were non-significant.

predator; $F(1, 14) = 0.238$), immobility ($F(1, 14) = 0.451$), locomotion ($F(1, 14) = 0.453$), and rearing or propping (leaning on arena wall; $F(1, 14) = 3.411$, $p = .083$) were all nonsignificant. IC subjects were significantly more likely than EC subjects to jump, usually in response to a near approach by the predator ($F(1, 14) = 12.023$, $p < .005$).

Time to escape under challenge was significantly lower in the EC rats. These times differed significantly from those for the IC rats ($F(1, 14) = 77.817$, $p < 0.001$); six IC rats failed to escape and were assigned

scores of 180 sec. The escape time distributions of EC and IC rats did not overlap. Behavior relative to the obstruction box during the three days was a good predictor of escape time under challenge (r = -0.795, $p <$ .001).

EXPERIMENT II

The results of experiment 1 demonstrate quite clearly that rats with experience in enriched and impoverished environments display a behavioral difference in response to apparent attack in an environment with which they have had opportunity to become familiar. The results cannot, however, rule out the possibility that the results of experiment 1 could be due to an environmentally induced difference in behavioral response to the stress of apparent attack rather than to intra-arena learning. In order to test this hypothesis, a second experiment was carried out eliminating the initial two days' experience in the arena.

METHOD

Animals

In Experiment 2, 12 naive male Sprague-Dawley rats were housed in standard colony conditions until the start of the experiment at 90 days of age.

Procedure

Procedures were identical to those used in Experiment 1, up until the point at which subjects were introduced into the arena. At this time, subjects were placed in the arena (configured as in Day 3 of Experiment 1) for three minutes, after which the motorized simulated predator emerged and began chasing the subject. Escape time was measured up to 180 seconds as in Experiment 1.

RESULTS

Behavioral profiles under challenge, again proportional to amount of time spent under challenge, are shown for IC and EC groups in Figure 2. For Experiment 2, all comparisons were non-significant, including relative amounts of time spent in investigation (F (1, 10) = 0.213), immobility (F (1, 10) = 1.497), locomotion (F (1, 14) = 0.403), and rearing or propping (F (1, 14) = 2.456), and jumping (F (1, 14) = 4.045, p = .07). As

in Experiment 1, EC rats escaped from the arena more quickly than IC ($F(1, 10) = 9.426$, $p < .05$); in fact, none of the IC subjects escaped within the 180 sec maximum time.

Trials were terminated at 180 seconds even though it was clear from pilot testing (in which rats that did not escape within 180 seconds did not escape at all, even given longer periods of being chased) that this would create a ceiling effect. Within 180 seconds the major questions of this study had been addressed; as a result, there was no further cause for continuing to stress the remaining rats. The only possible outcome that would embarrass the conclusion that inter-arena learning contributes significantly to later escape performance would be convergence of the groups with time in the arena; such convergence can be ruled out with these data in spite of the ceiling effect.

Combining the results of the two experiments yields a factorial experiment, with environmental condition and previous arena experience as between-subjects factors and escape time as the dependent measure. As shown in Figure 3, both the environmental condition and previous arena experience affected speed of escape. The effect of

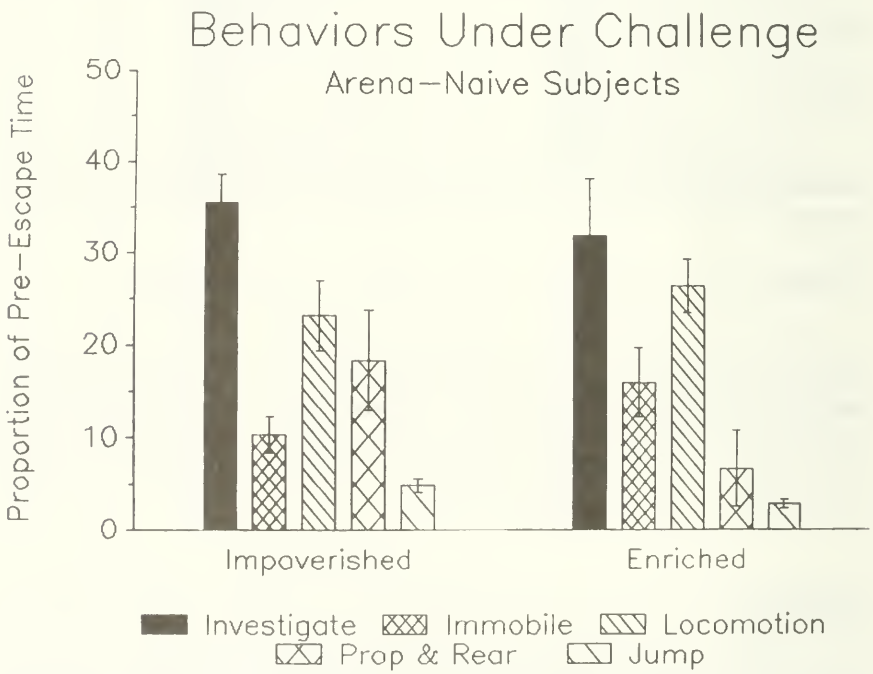

FIGURE 2. Behaviors under challenge, arena-naive subjects. Bars are mean proportion of pre-escape time (or 180 second session) engaged in these behaviors; bars indicate SEM for each measure. All comparisons were non-significant.

environmental condition was significant ($F(1, 24) = 64.46, p < .001$), as were the effects of previous experience in the arena ($F(1, 24) = 5.76, p < .05$) and the environment by experience interaction ($F(1, 24) = 4.67, p < .05$).

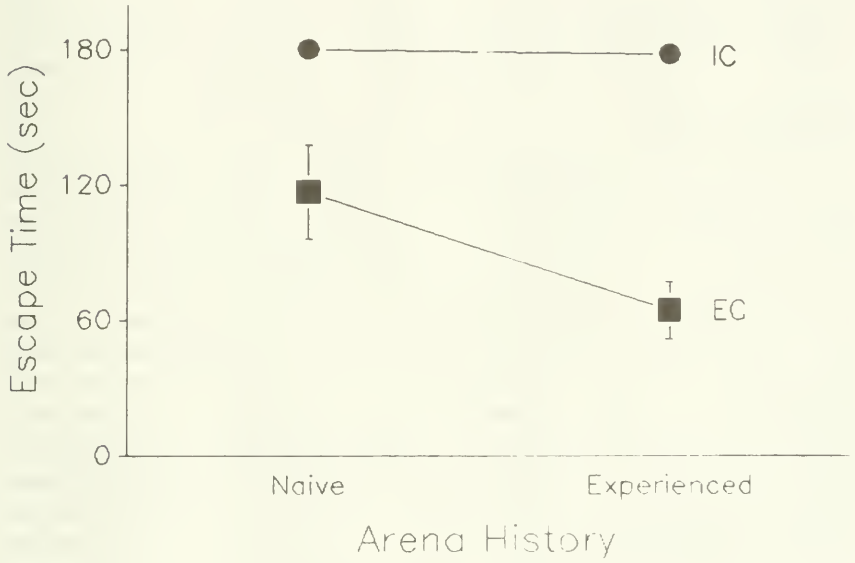

FIGURE 3. Summary figure: Effects of experience in arena and previous environmental treatment condition on escape time, to a maximum of 180 sec. Means for experienced animals are from data reported in the first experiment, naive animals from the second. Both main effects were significant, as was the condition-by-experience interaction. Standard error of the mean is shown for each EC group; standard error for each of the IC groups was smaller than the symbol used to plot the group mean.

DISCUSSION

The central question of this study was whether the particular behaviors employed in exploration influence the information gained as a result of that exploration. The behavior of the rats indicates that they do. These results demonstrate clearly that an animal can, by its own actions

or lack thereof, determine whether it gains information that may later have clear functional significance.

Although comparison of results from experiments run at different times opens the possibility of interference from extra-experimental variables, care was taken to minimize this risk: subjects for Experiments 1 and 2 were offspring from dams that were siblings, raised under identical colony conditions (approximately 40 days apart). Experimental procedures were identical other than for the experimental variable of pre-challenge arena experience. Limitations of equipment and of timing within the experiment prevented running this experiment initially as a two-factor design. In view of this, combination seems a valid tool for discussion.

There is a critical difference between these studies and the latent learning studies (Tolman & Honzik, 1930; Albert & Mah, 1972). In latent learning studies, the experimenters arranged situations in which subjects would, with very few exceptions, discover useful information; even random activity would lead to acquisition of this information. The behavior of the subjects themselves in those situations is of only minimal interest. In the experimental situation reported here, a random walk around the arena would not lead to discovery of the information that would later be useful. Instead, the subjects' behavior differed as a function of environmental history (a manipulation completed before subjects had access to the arena), and that behavioral difference determined whether the subjects gained information that was useful under later challenge.

The existence of an EC-IC difference in escape time on Day 1, when subjects have had little time to explore the arena, suggests that pre-existing differences in the behavioral hierarchies of subjects from IC and EC groups (previously documented in Renner and Rosenzweig, 1986, and Renner, 1987) do contribute to the results shown here. It is clear, however, that these pre-existing differences are not adequate as explanations of the EC-IC differences in escape time in Experiment 1 (arena-experienced subjects). In subjects given pre-challenge opportunity to explore the arena, no differences were observed prior to challenge in behaviors indicative of stress.

The only behaviors in which differences were observed were those behaviors relevant to predicting escape. In addition, behaviors under challenge do not show evidence that EC and IC subjects were differentially stressed by the apparent attack. Two relatively brief and procedurally identical experiences in the arena did not diminish the differences in escape time, and may have amplified them (although the obvious ceiling effect imposed by the 180 second trial length limit makes it impossible to evaluate this conclusion statistically). Changes in behavior that occurred after the subjects' introduction to the arena must have been the result of events occurring in the arena, which must in

turn be the product of the behaviors of the subjects themselves; this implies the operation of positive feedback.

Had the simulated predator in this experiment been a genuine threat, those animal whose previous actions had led to discovery of an escape route would presumably have had a substantially enhanced chance of surviving the incident. Those animals whose behaviors had not led to this discovery would presumably have been more likely to be caught and would therefore have had a reduced chance of survival.

As reported previously (Renner, 1987) and reinforced here, movements in space and interaction with inanimate features of the environment are separable aspects of exploratory behavior. Although much is known of learning and memory for space and spatial relationships, or knowledge of investigation and manipulation of objects is considerably less complete. If the specific details of an animal's behavioral organization exert substantial influence on what that individual gains from an instance of exploration, then detailed study of the behavioral topography of exploration may provide information important for a full understanding of its role in animal information processing.

The evidence provided by these experiments is consistent with data from other studies: exploratory behavior can lead to acquisition of information. This may, in fact, be its function, to create situations for information gathering. In addition, these results indicate that the behaviors employed in exploratory behavior can determine the information acquired during exploration. Changes in the characteristics of an individual's exploratory behavior can therefore exert significant influence on that individual's ability to behave adaptively.

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SHORT REPORT

CHIMPANZEE *(PAN TROGLODYTES)* MOTHERS' RESPONSE TO SEPARATION FROM INFANTS

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ABSTRACT: Three chimpanzee infants were separated from their mothers. The behavior of the mothers was monitored before and after separation. Data were equally divided between pre- and post-separation observation periods. The mothers exhibited significantly reduced levels of play and significantly more time spent in proximity to an older offspring after they were permanently separated from their infants. No other recorded behaviors were significantly altered. The mothers exhibited individual differences immediately following the separation. The findings are consistent with other studies that noted the relatively mild maternal reactions to infant separation and the attenuating effect of familiar conspecifics in the post-separation environment.

INTRODUCTION

No report of great ape maternal response to infant separation has been published. The extensive study of social separation in nonhuman primates has emphasized the response of infants removed from their mothers. A few experimenters have described the behavior of adolescent or young adult macaques after such separation (Bowden and McKinney, 1972; Suomi et al., 1975) and the response of monkey mothers to separation from their infants (Hinde and McGinnis, 1977; Jensen, 1968; Jensen and Tolman, 1962; Suomi et al., 1983). The purpose of the present pilot study was to document the response of chimpanzee mothers to separation, identifying evidence of "protest" or "despair" (under Bowlby's scheme (1960)) as the mother continued to live in a familiar social group.

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METHODS

Animals

Three competent mother chimpanzees *(Pan troglodytes)* between 19 and 21 years old were observed. Each mother had an infant between 21 and 23 months of age that was separated from her during the study and an older offspring between five and six years of age that had lived with her continually. Each chimpanzee lived in a stable social group consisting of adults of both sexes and their offspring in similar, 22-m outdoor corrals containing climbing structures, large cement culverts, movable objects, and natural, grassy ground cover. The facilities and colony management have been described (Riddle et al., 1982).

Procedure

Data were collected for three weeks prior to and three weeks following the mother-infant separation. (The separations were completed for the purposes of another study). Twenty 15-min observation sessions were conducted for each mother during each of the two experimental phases, totalling 30 hours of data collection. Eleven behaviors representing seven different behavioral categories (agonism, prosocial behavior, locomotion, inactivity, vocalization, abnormal and other behaviors) were recorded at 10-second intervals using a scan-sampling technique. The category of prosocial behavior was composed of grooming and playing. The vocalizations recorded were pant grunts, pant hoots, food calls and whimpering. The age and sex of each animal interacting with the focal animal at the sampling point was recorded in addition to all animals within 1 m of the focal animal.

On the day of separation the three mothers, with their infants, were removed from their social groups and anesthetized with ketamine hydrochloride. When alert, they were fed a small meal and then reintroduced without their infants to their social groups approximately 7 hours after the initial separation. The first post-separation observation sessions was conducted upon their re-entry.

Analysis

Data were analyzed by the nonparametric sign test for related samples to detect differences between pre- and post-separation levels in each of the seven behavioral categories and in the proximity information. Statistical comparisons were made between pairs of data collection sessions composed of one pre-test and one post-test session.

RESULTS

Sign test results indicated that the animals displayed significantly lower levels of play ($p < .001$) and spent significantly more time in proximity to their older offspring ($p = .003$) in the post-separation phase. No other recorded behaviors or states of proximity were significantly changed.

Some individual differences in the behavior were obvious when the data were examined. One mother vocalized twice as much in the first session after separation than she did in any other session. All of these 32 instances of vocalizing (of 90 possible) were whimpering. She also engaged in self-slapping (during 5 of 90 data points) and rocked (during 13 of the 90 data points). However, by the following day her behavior was not distinguishable from her pre-separation behavior. None of the others had been observed to self-slap, rock, or whimper in the pre-separation phase. One other mother appeared to look toward the room that housed the recently separated infants (during 12 of the 90 data points). Though visual contact was not possible, she may have heard infant vocalizations, but neither she nor the third mother reacted like the subject described earlier.

During the post-separation phase two of the three chimpanzees were not observed playing (illustrated by the significant sign test result), but the third mother's level of play doubled. All of her post-separation play was recorded late in the study, after the seventeenth post-separation data collection session. Prior to separation from her infant, this mother had divided her play between her infant and her older offspring (57% and 43% respectively). After the separation, 93% of her play was with her older offspring. In contrast, greater percentages of the other mothers' play was with their infants (77% and 93%), and smaller percentages with their older offspring (3.5% and 4.5%).

DISCUSSION

The results indicate little evidence of an extended "protest" and no evidence of "despair" (Bowlby, 1960) by the three chimpanzee mothers after permanent separation from their infants. This finding is consistent with studies of monkey mothers (Hinde and McGinnis, 1977; Jensen, 1968; Suomi et al., 1983), although evidence of a brief period of agitation has been reported (Jensen, 1968). Individual differences were apparent among the three chimpanzees. Two of the three displayed no protest or agitation reaction. The third showed her highest levels of vocalizing, self-slapping, and rocking during the session immediately following the separation. This protest was not displayed beyond the first day after

separation. No mother showed evidence of despair as might have been indicated by high levels of inactivity. The mother that displayed the protest was the only one that showed an increased level of play during the post-separation phase, which can be interpreted as additional evidence for a lack of despair. The two other chimpanzees did not play in the post-separation phase, perhaps because their infants had been their predominant play partners while about half of the other mother's pre-separation play was with her older son.

The presence of familiar group members in the post-separation environment may have attenuated the mothers' reactions to separation from their infants, as has been found in other separation studies (Bard and Nadler, 1983; Codner and Nadler, 1984; Suomi et al., 1975). The presence of the older offspring may have been particularly important; in significantly more instances the mothers were in proximity to their older offspring in the post-separation phase.

In conclusion, a cross-species similarity in maternal response is suggested by the agreement between the results of this study and those utilizing macaques. The individual variation described in the severity of the short-duration protest reaction, however, may justify further investigation in great apes.

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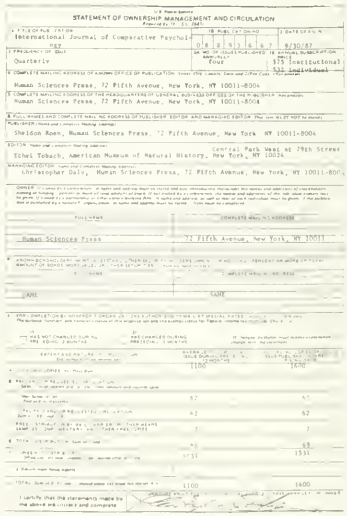

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